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Patentanmeldung Nr. | **Patent application No.** | **Demande de brevet n°**

85905513.9

München, den
Munich,
Munich, le 27. 10. 92

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: 85905513.9

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Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention: ENVELOPE ANTIGENS OF LYMPHADENOPATHY ASSOCIATED VIRUS AND THEIR APPLICATIONS

Anmeldetag:
Date of filing:
Date de dépôt: 18.10.85

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EP 85/00548

THE PATENT OFFICE,
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PCT/EP85/00548

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19/11/84 B3662 PAT*** 10.00

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REQUEST FOR GRANT OF A PATENT

8429099

THE GRANT OF A PATENT IS REQUESTED BY THE UNDERSIGNED ON THE BASIS OF THE PRESENT APPLICATION

I Agent's Reference JJD/EAF/26804

II Title of Invention CLONED DNA SEQUENCES RELATED TO THE GENOMIC RNA OF LYMPHADENOPATHY-ASSOCIATED VIRUS (LAV) AND PROTEINS ENCODED BY SAID LAV GENOMIC RNA.

III Applicant or Applicants *(See note 2)*

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~~(a) The applicant(s) is/are the sole inventor(s)~~

or

(b) A statement on Patents Form No. 7/77 is/will be furnished

V Name of Agent (if any) *(See note 4)* Reddie & Grose | ADP CODE NO

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London WC1X 8PL

VII Declaration of Priority (See note 6)

Country	Filing date	File number

THE PATENT OFFICE
16 NOV 1984
25 SOUTHAMPTON BUILDINGS
LONDON WC2A 1AY

VIII The Application claims an earlier date under Section 8(3), 12(6), 15(4), 37(4) (See note 7)

Section No.

Earlier application or patent number and filing date

IX Check List *(To be filled in by applicant or agent)*

A The application contains the following number of sheet(s)		B The application as filed is accompanied by:–	
1 Request	1 Sheet(s)	1 Priority document	No
2 Description	17 Sheet(s)	2 Translation of priority document	No
3 Claim(s)	2 Sheet(s)	3 Request for Search	No
4 Drawing(s)	26 Sheet(s)	4 Statement of Inventorship and Right to Apply	No
5 Abstract	0 Sheet(s)	5	

X It is suggested that Figure No 1 of the drawings (if any) should accompany the abstract when published.

XI Signature *(See note 8)*

Reddie & Grose, Agents for the Applicant(s)

NOTES:

1. This form, when completed, should be brought or sent to the Patent Office together with the prescribed fee and two copies of the description of the invention, and of any drawings.
2. Enter the name and address of each applicant. Names of individuals should be indicated in full and the surname or family name should be underlined. The names of all partners in a firm must be given in full. Bodies corporate should be designated by their corporate name and the country of incorporation and, where appropriate, the state of incorporation within that country should be entered where provided. Full corporate details, eg "a corporation organised and existing under the laws of the State of Delaware, United States of America," trading styles, eg "trading as xyz company", nationality, and former names, eg "formerly [known as] ABC Ltd." are not required and should not be given. Also enter applicant(s) ADP Code No. (if known).
3. Where the applicant or applicants is/are the sole inventor or the joint inventors, the declaration (a) to that effect at IV should be completed, and the alternative statement (b) deleted. If, however, this is not the case the declaration (a) should be struck out and a statement will then be required to be filed upon Patent Form No 7/77.
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6. The declaration of priority at VII should state the date of the previous filing and the country in which it was made and indicate the file number, if available.
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Cloned DNA sequences related to the genomic RNA of lymphadenopathy-associated-virus (LAV) and protsins encoded by said LAV genomic RNA

The invention relates to cloned DNA sequences indistinguishable from genomic RNA and DNA of lymphadenopathy-associated virus (LAV), a process for their preparation and their uses. It relates more particularly to stable probes including a DNA sequence which can be used for the detection of the LAV virus or related viruses or DNA proviruses in any medium, particularly biological samples containing any of them. The invention also relates to polypeptides, whether glycosylated or not, encoded by said DNA sequences.

Lymphadenopathy-associated virus (LAV) is a human retrovirus first isolated from the lymph node of a homosexual patient with lymphadenopathy syndrome, frequently a prodrome or a benign form of acquired immune deficiency syndrome (AIDS). Subsequently other LAV isolates have been recovered from patients with AIDS or pre-AIDS. All available data are consistent with the virus being the causative agent of AIDS.

A method for cloning such DNA sequences has already been disclosed in British Patent Application Nr. 84 23659 filed on September 19, 1984. Reference is hereafter made to that application as concerns subject matter in common with the further improvements to the invention disclosed herein.

The present invention aims at providing additional new means which should not only also be useful for the detection of LAV or related viruses (hereafter more generally referred to as "LAV viruses"), but also have more versatility, particularly in detecting specific parts of the genomic DNA of said viruses whose expression products are not always directly detectable by immunological methods.

The present invention further aims at providing

polypeptides containing sequences in common with polypeptides encoded by the LAV genomic RNA. It relates even more particularly to polypeptides comprising antigenic determinants included in the proteins encoded and expressed by the LAV genome occuring in nature. An additional object of the invention is to further provide means for the detection of proteins related to LAV virus, particularly for the diagnosis of AIDS or pre-AIDS or, to the contrary, for the detection of antibodies against the LAV virus or proteins related therewith, particularly in patients afflicted with AIDS or pre-AIDS or more generally in asymtomatic carriers and in blood-related products. Finally the invention also aims at providing immunogenic polypeptides, and more particularly protective polypeptides for use in the preparation of vaccine compositions against AIDS or related syndroms.

The present invention relates to additional DNA fragments, hybridizable with the genomic RNA of LAV as they will be disclosed hereafter, as well as with additional cDNA variants corresponding to the whole genomes of LAV viruses. It further relates to DNA recombinants containing said DNAs or cDNA fragments.

The invention relates more particularly to a cDNA variant corresponding to the whole of LAV retroviral genomes, which is characterized by a series of restriction sites in the order hereafter (from the 5' end to the 3' end).

The coordinates of the successive sites of the whole LAV genome (restriction map) are indicated hereafter too, with respect to the Hind III site (selected as of coordinate 1) which is located in the R region. The coordinates are estimated with an accuracy of ± 200 bp :

Hind III	0
Sac I	50
Hind III	520
Pst I	800
Hind III	1 100

Bgl II	1 500
Kpn I	3 500
Kpn I	3 900
Eco RI	4 100
Eco RI	5 300
Sal I	5 500
Kpn I	6 100
Bgl II	6 500
Bgl II	7 600
Hind III	7 850
Bam HI	8 150
Xho I	8 600
Kpn I	8 700
Bgl II	8 750
Bgl II	9 150
Sac I	9 200
Hind III	9 250

Another DNA variant according to this invention optionally contains an additional Hind III approximately at the 5 550 coordinate.

Reference is further made to fig. 1 which shows a more detailed restriction map of said whole-DNA (λJ19).

An even more detailed nucleotidie sequence of a preferred DNA according to the invention is shown in fig. 4-12 hereafter.

The invention further relates to other preferred DNA fragments which will be referred to hereafter.

Additional features of the invention will appear in the course of the non-limitative disclosure of additional features of preferred DNAs of the invention, as well as of preferred polypeptides according to the invention. Reference will further be had to the drawings in which :

- fig. 1 is the restriction map of a complete LAV genome (clone λJ19) ;
- figs. 2 and 3 show diagrammatically parts of the three

possible reading phases of LAV genomic RNA, including the open reading frames (ORF) apparent in each of said reading phases ;

- figs. 4-12 show the successive nucleotidic sequences of a complete LAV genome. The possible peptidic sequences in relation to the three possible reading phases related to the nucleotidic sequences shown are also indicated ;
- figs. 13-18 reiterate the sequence of part of the LAV genome containing the genes coding for the enveloppe proteins, with particular boxed peptidic sequences which corresponds to groups which normally carry glycosyl groups.

The sequencing and determination of sites of particular interest was carried out on a phage recombinant corresponding to λJ19 disclosed in the abovesaid British Patent application Nr. 84 23659. A method for preparing it is disclosed in that application.

The whole recombinant phage DNA of clone λJ19 (disclosed in the earlier application) was sonicated according to the protocol of DEININGER (1983), Analytical Biochem. 129, 216. the DNA was repaired by a Klenow reaction for 12 hours at 16°C. The DNA was electrophoresed through 0.8 % agarose gel and DNA in the size range of 300-600 bp was cut out and electroeluted and precipitated. Resuspended DNA (in 10 mM Tris, pH 8 ; 0,1 mM EDTA) was ligated into M13mp8 RF DNA (cut by the restriction enzyme SmaI and subsequently alkaline phosphated), using T4 DNA- and RNA-ligases (Maniatis T et al (1982) - Molecular cloning - Cold Spring Harbor Laboratory). An E. coli strain designated as TG1 was used for further study. This strain has the following genotype :

Δlac pro, supE, thi.F'traD36, proAB, $lacI^q$, ZΔM15,r^-

This E. coli TGI strain has the peculiarity of enabling recombinants to be recognized easily. The blue colour of the cells transfected with plasmids which did

not recombine with a fragment of LAV DNA is not modified. To the contrary cells transfected by a recombinant plasmid containing a LAV DNA fragment yield white colonies. The technique which was used is disclosed in Gene (1983), 26, 101.

This strain was transformed with the ligation mix using the Hanahan method (Hanahan D (1983) J. Mol. Biol. 166, 557). Cells were plated out on tryptone-agarose plate with IPTG and X-gal in soft agarose. White plaques were either picked and screened or screened directly in situ using nitrocellulose filters. Their DNAs were hybridized with nick-translated DNA inserts of pUC18 Hind III subclones of λJ19. this permitted the isolation of the plasmids or subclones of λ which are identified in the table hereafter. In relation to this table it should also be noted that the designation of each plasmid is followed by the deposition number of a cell culture of E. coli TGI containing the corresponding plasmid at the "Collection Nationale des Cultures de Micro-organismes" (C.N.C.M.) of the Pasteur Institute in Paris, France. A non-transformed TGI cell line was also deposited at the C.N.C.M. under Nr. I-364. All these deposits took place on November 15, 1984. The sizes of the corresponding inserts derived from the LAV genome have also been indicated.

TABLE

Essential features of the recombinant plasmids

- pJ19 - 1 plasmid (I-365) 0.5 kb

Hind III - Sac I - Hind III

- pJ19 - 17 plasmid (I-367) 0.6 kb

Hind III - Pst 1 - Hind III

- pJ19 - 6 plasmid (I-366) 1.5 kb

Hind III (5')
Bam HI
Xho I
Kpn I
Bgl II
Sac I (3')
Hind III

- pJ19-13 plasmid (I-368) 6.7 kb

Hind III (5')
Bgl II
Kpn I
Kpn I
Eco RI
Eco RI
Sal I
Kpn I
Bgl II
Bgl II
Hind III (3')

Positively hybridizing M13 phage plates were grown up for 5 hours and the single-stranded DNAs were extracted.

M13mp8 subclones of λJ19 DNAs were sequenced according to the dideoxy method and technology devised by Sanger et al (Sanger et al (1977), Proc. Natl. Acad. Sci. USA, 74, 5463 and M13 cloning and sequencing handbook, AMERSHAM (1983). the 17-mer oligonucleotide primer α-^{35}SdATP (400Ci/mmol, AMERSHAM), and 0.5X-5X buffer gradient gels (Biggen M.D. et al (1983, Proc. Natl. Acad. Sci. USA, 50, 3963) were used. Gels were read and put into the computer under the programs of Staden (Staden R. (1982), Nucl. Acids Res. 10, 4731). All the appropriate references and methods can be found in the AMERSHAM M13 cloning and sequencing handbook.

The complete sequence of λJ19 was deduced from the experiments as further disclosed hereafter.

Figs. 4-12 provide the DNA nucleotidie sequence of the complete genome of LAV. The numbering of the nucleotides starts from a left most Hind III restriction site (5'AAG..) of the restriction map. The numbering occurs in tens whereby the last zero number of each of the numbers occuring on the drawings is located just below the nucleotide corresponding to the nucleotides designated. I.e. the nucleotide at position 10 is T, the nucleotide at position 20 is C, etc..

Above each of the lines of the successive nucleotidic sequences there are provided three lines of single letters corresponding to the aminoacid sequence deduced from the DNA sequence (using the genetic code) for each at the three reading phases, whereby said single letters have the following meanings.

A : alanine
R : arginine
K : lysine
H : histidine
C : cysteine

M : méthionine
W : tryptophan
F : phenylalanine
Y : tyrosine
L : leucine
V : valine
I : isoleucine
G : glycine
T : thréonine
S : serine
E : glutamic acid
D : Aspartic acid
N : asparagine
Q : glutamine
P : proline.

The asterik signs "*" correspond to stop codons (i.e. TAA, TAG and TGA).

Starting above the first line of the DNA nucleotidic sequence of fig. 4 the three reading phases are respectively marked "1", "2", "3", on the left handside of the drawing. The same relative presentation of the three theoritical reading phases is then used all over the successives lines of the LAV nucleotidic sequence.

Figs. 2 and 3 provide a diagrammatized representation of the lengths of the successive open reading frames corresponding to the successive reading phases (also referred to by numbers "1", "2" and "3" appearing in the left handside part of fig. 2). The relative positions of these open reading frames (ORF) with respect to the nucleotidic structure of the LAV genome is referred to by the scale of numbers representative of the respective positions of the corresponding nucleotides in the DNA sequence. The vertical bars correspond to the positions of the corresponding stop codons.

1) <u>The "gag gene" (or ORF-gag)</u>

The "gag gene" codes for core proteins.

Particularly it appears that a genomic fragment (ORF-gag) thought to code for the core antigens including the p25, p18 and p13 proteins is located between nucleotidic position 236 (starting with 5' CTA GCG GAG 3') and nucleotidic position 1759 (ending by CTCG TCA CAA 3'). The structure of the peptides or proteins encoded by parts of said ORF is deemed to be that corresponding to phase 2.

The methionine aminoacid "M" coded by the ATG at position 260-262 is the probable initiation methionine of the gag protein precursor. The end of ORF-gag and accordingly of gag protein appears to be located at position 1759.

The beginning of p25 protein, thought to start by a P-I-V-Q-N-I-Q-G-Q-M-V-H .... aminoacid sequence is thought to be coded for by the nucleotidic sequence CCTATA..., starting at position 655.

Hydrophilic peptides in the gag open reading frame are identified hereafter. They are defined starting from aminoacid 1 = Met (M) coded by the ATG starting from 260-2 in the LAV DNA sequence.

Those hydrophilic peptides are

12-32	aminoacids	inclusive
37-46	"	"
49-79	"	"
88-153	"	"
158-165	"	"
178-188	"	"
200-220	"	"
226-234	"	"
239-264	"	"
288-331	"	"
352-361	"	"
377-390	"	"
399-432	"	"
437-484	"	"
492-498	"	"

The invention also relates to any combination of these peptides.

2) The "pol gene" (or ORF-pol)

Figs. 4-12 also show that the DNA fragments extending from nucleotidic position 1555 (starting with 5'TTT TTT ....3' to nucleotidic position 5086 is thought to correspond to the pol gene. The polypeptidic structure of the corresponding polypeptides is deemed to be that corresponding to phase 1. It stops at position 4583 (end by 5'G GAT GAG GAT 3').

These genes are thought to code for the virus polymerase or reverse transcriptase.

3) The envelope gene (or ORF-env)

The DNA sequence thought to code for envelope proteins is thought to extend from nucleotidic position 5670 (starting with 5'AAA GAG GAG A....3') up to nucleotidic position 8132 (ending by ....A ACT AAA GAA 3'). Polypeptidic structures of sequences of the envelope protein correspond to those read according to the "phase 3" reading phase.

The start of env transcription is thought to be at the level of th ATG codon at positions 5691-5693.

Additional feature of the envelope protein coded by the env genes appear on figs. 13-18. These are to be considered as paired figs. 13 and 14 ; 15 and 16 ; 17 and 18 respectively.

It is to be mentioned that because of format difficulties.

Fig. 14 overlaps to some extent with fig. 13.

Fig. 16 overlaps to some extent with fig. 15.

Fig. 18 overlaps to some extent with fig. 17.

Thus for instance figs. 13 and 14 must be considered together. Particularly the sequence shown on the first line on the top of fig. 13 overlaps with the sequence shown on the first line on the top of fig. 14. In other words the starting of the reading of the successive

sequences of the env gene as represented in figs. 13-18 involves first reading the first line at the top of fig. 13 then proceeding further with the first line of fig. 14. One then returns to the beginning of the second line of fig. 13, then again further proceed with the reading of the second line of page 14, etc... The same observations then apply to the reading of the paired figs. 15 and 16, and paired figs. 17 and 18, respectively.

The locations of neutralizing epitopes are further apparent in figs. 13-18. reference is more particularly made to the boxed groups of three letters included in the aminoacid sequences of the envelope proteins (reading phase 3) which can be designated generally by the formula N-X-S or N-X-T, wherein X is any other possible aminoacid. Thus the initial protein product of the env gene in a glycoprotein of molecular weight in excess of 91,000. These groups are deemed to generally carry glycosylated groups. These N-X-S and N-X-T groups with attached glycosylated groups form together hydrophylic regions of the protein and are deemed to be located at the periphery of and to be exposed outwardly with respect to the normal conformation of the proteins. Consequently they are considered as being epitopes which can efficiently be brought into play in vaccine compositions.

The invention thus concerns with more particularity peptide sequences included in the env-proteins and excizable therefrom (or having the same aminoacid structure), having sizes not exceeding 200 aminoacids.

Preferred peptides of this invention (referred to hereafter as a, b, c, d, e, f) are deemed to correspond to those encoded by the nucleotide sequences which extend respectively between the following positions :

a) from about 6095 to about 6200
b) " " 6260 " " 6310
c) " " 6390 " " 6440
d) " " 6485 " " 6620

e) " " 6860 " " 6930

f) " " 7535 " " 7630

Other hydrophilic peptides in the env open reading frame are identified hereafter. they are defined starting from

aminoacid 1 = lysine (K) coded by the AAA at position 5670-2 in the LAV DNA sequence.

These hydrophilic peptides are

8-23 aminoacids inclusive
63-78 " "
82-90 " "
97-123 " "
127-183 " "
197-201 " "
239-294 " "
300-327 " "
334-381 " "
397-424 " "
486-500 " "
510-523 " "
551-577 " "
594-603 " "
621-630 " "
657-679 " "
719-758 " "
780-803 " "

The invention also relates to any combination of these peptides.

4) <u>The other ORF</u>

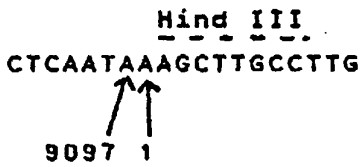
The invention further concerns DNA sequences which provide open reading frames defined as ORF-Q, ORF-R and as "1", "2", "3", "4", "5", the relative position of which appears more particularly in figs. 2 and 3.

These ORFs have the following locations :

ORF-Q	phase 1	start 4478	stop 5086
ORF-R	" 2	" 8249	" 8896

ORF-1	"	1	"	5029	"	5316
ORF-2	"	2	"	5273	"	5515
ORF-3	"	1	"	5383	"	5616
ORF-4	"	2	"	5519	"	5773
ORF-5	"	1	"	7966	"	8279

The LTR (long terminal repeats) can be defined as lying between position 8560 and position 160 (end extending over position 9097/1). As a matter of fact the end of the genome is at 9097 and, because of the LTR structure of the retrovirus, links up with the beginning of the sequence :



The invention concerns more particularly all the DNA fragments which have been more specifically referred to hereabove and which correspond to open reading frames. It will be understood that the man skilled in the art will be able to obtain them all, for instance by cleaving an entire DNA corresponding to the complete genome of a LAV species, such as by cleavage by a partial or complete digestion thereof with a suitable restriction enzyme and by the subsequent recovery of the relevant fragments. The different DNAs disclosed in the earlier mentioned British Application can be resorted to also as a source of suitable fragments. The techniques disclosed hereabove for the isolation of the fragments which were then included in the plasmids referred to hereabove and which were then used for the DNA sequencing can be used.

Of course other methods can be used. Some of them have been examplified in the earlier British Application. reference is for instance made to the following methods.

a) DNA can be transfected into mammalian cells with appropriate selection markers by a variety of techniques, calcium phosphate precipitation, polyethylene

glycol, protoplast-fusion, etc..

b) DNA fragments corresponding to genes can be cloned into expression vectors for E. coli , yeast- or mammalian cells and the resultant proteins purified.

c) The provival DNA can be "shot-gunned" (fragmented) into procaryotic expression vectors to generate fusion polypeptides. Recombinant producing antigenically competent fusion proteins can be identified by simply screening the recombinants with antibodies against LAV antigens .

The invention also relates more specifically to cloned probes which can be made starting from any DNA fragment according to this invention, thus to recombinant DNAs containing such fragments, particularly any plasmids amplifiable in procaryotic or eucaryotic cells and carrying said fragments.

Using the cloned DNA fragments as a molecular hybridization probe - either by marking with radionucleotides or with fluorescent reagents - LAV virion RNA may be detected directly in the blood, body fluids and blood products (e.g. of the antihemophylic factors such as Factor VIII concentrates) and vaccines, i.e. hepatitis B vaccine. It has already been shown that whole virus can be detected in culture supernatants of LAV producing cells. A suitable method for achieving that detection comprises immobilizing virus onto said a support e.g. nitrocellulose filters, etc., disrupting the virion and hybridizing with labelled (radiolabelled or "cold" fluorescent- or enzyme-labelled) probes. Such an approach has already been developed for Hepatitis B virus in peripheral blood (according to SCOTTO J. et al. Hepatology (1983), 3, 379-384).

Probes according to the invention can also be used for rapid screening of genomic DNA derived from the tissue of patients with LAV related symptoms, to see if the proviral DNA or RNA is present in host tissue and other

tissues.

A method which can be used for such screening comprise the following steps : extraction of DNA from tissue, restriction enzyme cleavage of said DNA, electrophoresis of the fragments and Southern blotting of genomic DNA from tissues, subsequent hybridization with labelled cloned LAV provival DNA. Hybridization in situ can also be used.

Lymphatic fluids and tissues and other non-lymphatic tissues of humans, primates and other mammalian species can also be screened to see if other evolutionnary related retrovirus exist. The methods referred to hereabove can be used, although hybridization and washings would be done under non stringent conditions.

The DNA according to the invention can be used also for achieving the expression of LAV viral antigens for diagnostic purposes.

The invention also relates to the polypeptides themselves which can be expressed by the different DNAs of the inventions, particularly by the ORFs or fragments thereof, in appropriate hosts, particularly procaryotic or eucaryotic hosts, after transformation thereof with a suitable vector previously modified by the corresponding DNAs.

These polypeptides can be used as diagnostic tools, particularly for the detection of antibodies in biological media, particularly in sera or tissues of persons afflicted with pre-AIDS or AIDS, or simply carrying antibodies in the absence of any apparent disorders. Conversely the different peptides according to this invention can be used themselves for the production of antibodies, preferably monoclonal antibodies specific of the different peptides respectively. For the production of hybridomas secreting said monoclonal antibodies conventional production and screening methods are used. These monoclonal antibodies, which themselves are part of

the invention then provide very useful tools for the identification and even determination of relative proportions of the different polypeptides or proteins in biological samples, particularly human samples containing LAV or related viruses.

Thus all of the above peptides can be used in diagnostics as sources of immunogens or antigens free of viral particles, produced using non-permissive systems, and thus of little or no biohazard risk.

The invention further relates to the hosts (procaryotic or eucaryotic cells) which are transformed by the above mentioned recombinants and which are capable of expressing said DNA fragments.

Finally it also relates to vaccine compositions whose active principle is to be constituted by any of the expressed antigens, i.e. whole antigens, fusion polypeptides or oligopeptides in association with a suitable pharmaceutical or physiologically acceptable carrier.

Preferably the active principles to be considered in that field consist of the peptides containing less than 250 aminoacid units, preferably less than 150 as deducible for the complete genomes of LAV, and even more preferably those peptides which contain one or more groups selected from N-X-S and N-X-T as defined above. Preferred peptides for use in the production of vaccinating principles are peptides (a) to (f) as defined above. By way of example having no limitative character, there may be mentioned that suitable dosages of the vaccine compositions are those which enable administration to the host, particularly human host ranging from 10 to 500 micrograms per kg, for instance 50 to 100 micrograms per kg.

For the purpose of clarity figs. 19 to 26 are added. reference may be made thereto in case of difficulties of reading blurred parts of figs. 4 to 12.

Needless to say that figs. 19-26 are merely a reiteration of the whole DNA sequence of the LAV genoma.

Finally the invention also concerns vectors for the transformation of eucaryotic cells of human origin, particularly lymphocytes, the polymerases of which are capable of recognizing the LTRs of LAV. Particularly said vectors are characterized by the presence of a LAV LTR therein, said LTR being then active as a promoter enabling the efficient transcription and translation in a suitable host of the above defined, of a DNA insert coding for a determined protein placed under its controls.

Needless to say that the invention extends to all variants of genomes and corresponding DNA fragments (ORFs) having substantially equivalent properties, all of said genomes belonging to retroviruses which can be considered as equivalents of LAV.

CLAIMS

1. A DNA fragment of LAV extending from nucleotide position 236 to nucleotide position 1759.

2. A DNA fragment of LAV extending from nucleotide position 1555 to nucleotide position 5086.

3. A DNA fragment of LAV extending from nucleotide position 5670 to nucleotide position 8132.

4. A vector containing a DNA fragment according to any of claims 1 to 3.

5. Peptide corresponding to any of those encoded by the nucleotide sequences which extend respectively between the following positions :

a) from about 6095 to about 6200
b) " " 6260 " " 6310
c) " " 6390 " " 6440
d) " " 6485 " " 6620
e) " " 6860 " " 6930
f) " " 7535 " " 7630

6. Peptide characterized by a sequence of amino-acids deducible from LAV DNA the terminal aminoacids of which extend between the following positions with respect to the lysine (position 1) coded by the AAA at position 5670-5672 in the LAV DNA.

8-23 aminoacids inclusive
63-78 " "
82-90 " "
97-123 " "
127-183 " "
197-201 " "
239-294 " "
300-327 " "
334-381 " "
397-424 " "
466-500 " "
510-523 " "
551-577 " "

594-603 " "
621-630 " "
657-679 " "
719-758 " "
780-803 " "

or any combination of these peptides.

7. Peptide corresponding to the aminoacid sequences deducible from LAV DNA and the terminal aminoacids of which are positionned at the positions hereafter counted from the Met at position 1 coded by the ATG sequence at nucleotide positions 260-2 :

12-32 aminoacids inclusive
37-46 " "
49-79 " "
88-153 " "
158-165 " "
178-188 " "
200-220 " "
226-234 " "
239-264 " "
288-331 " "
352-361 " "
377-390 " "
399-432 " "
437-484 " "
492-498 " "

and combination of said peptides.

8. Diagnostic means containing any of the DNA fragments of any of claims 1 to 3.

9. Diagnostic means containing any of the peptides of any of claims 4 to 6.

10. Vaccine compositions containing any of the peptides according to any of claims 4 to 6 in association with a pharmaceutical vehicle.

End of transmission

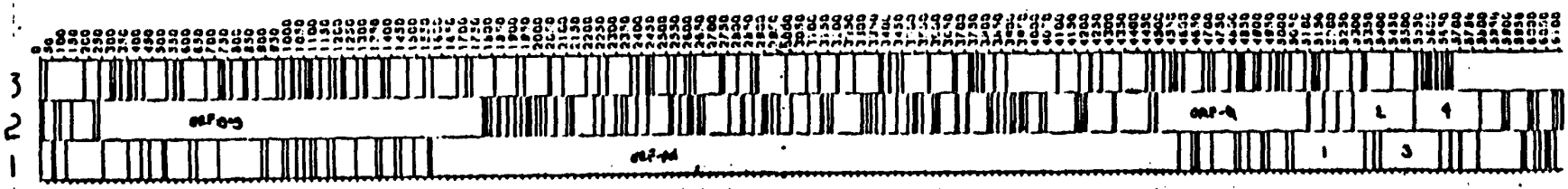
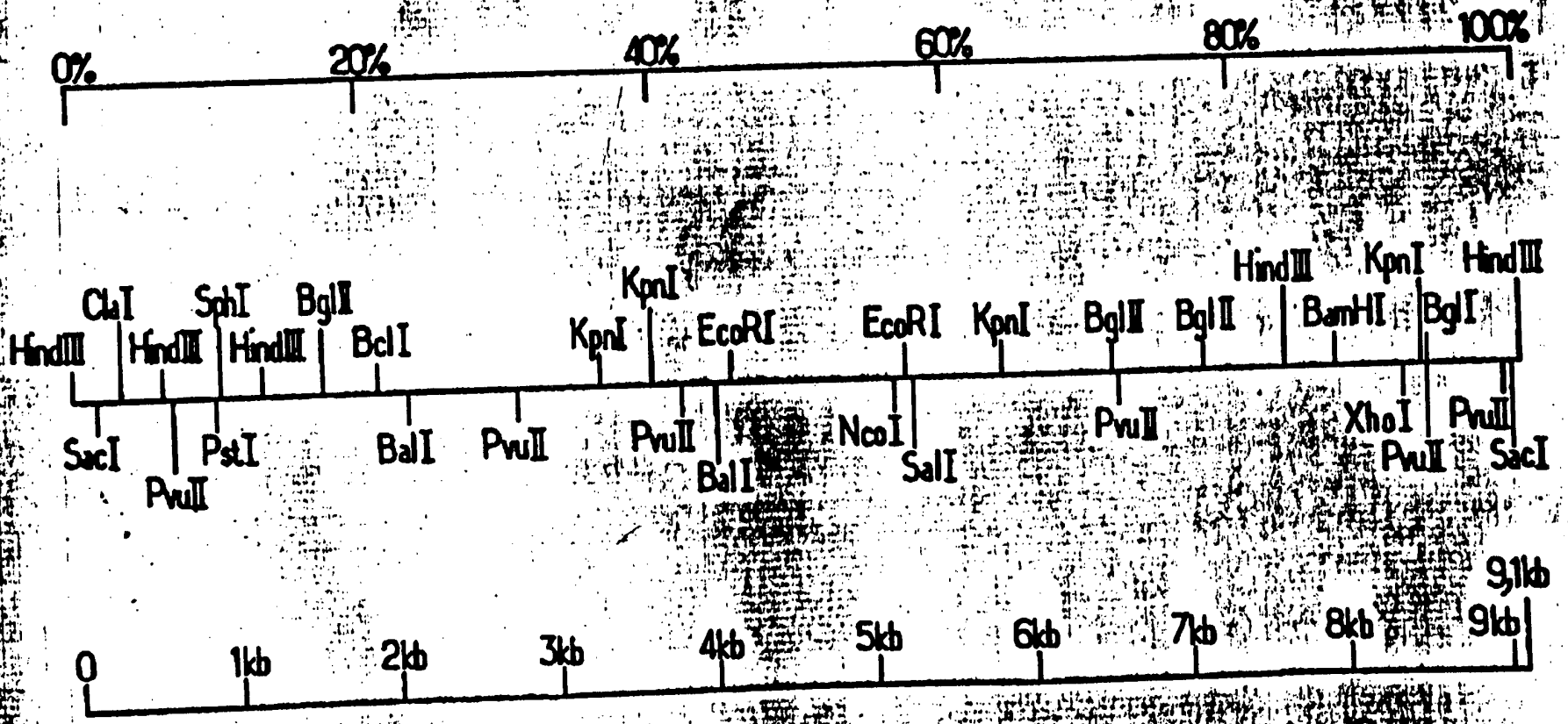


Fig. 2

FIG.1.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60%
80%
100%
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ClaI
HindIII
SphI
HindIII
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KpnI
KpnI
EcoRI
EcoRI
KpnI
BglII
BglII
HindIII
BamHI
KpnI
BglI
HindIII
SacI
PvuII
PstI
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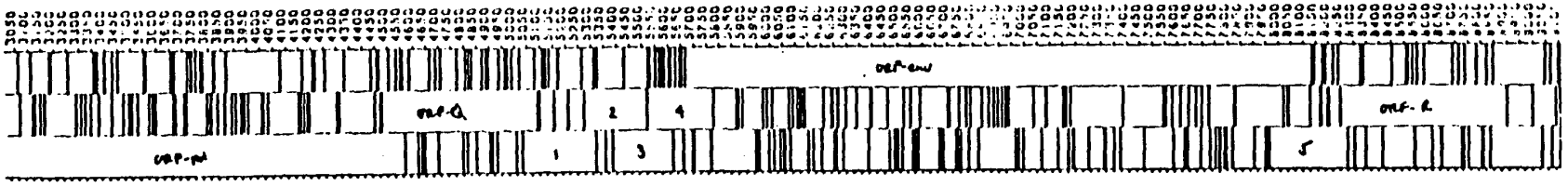
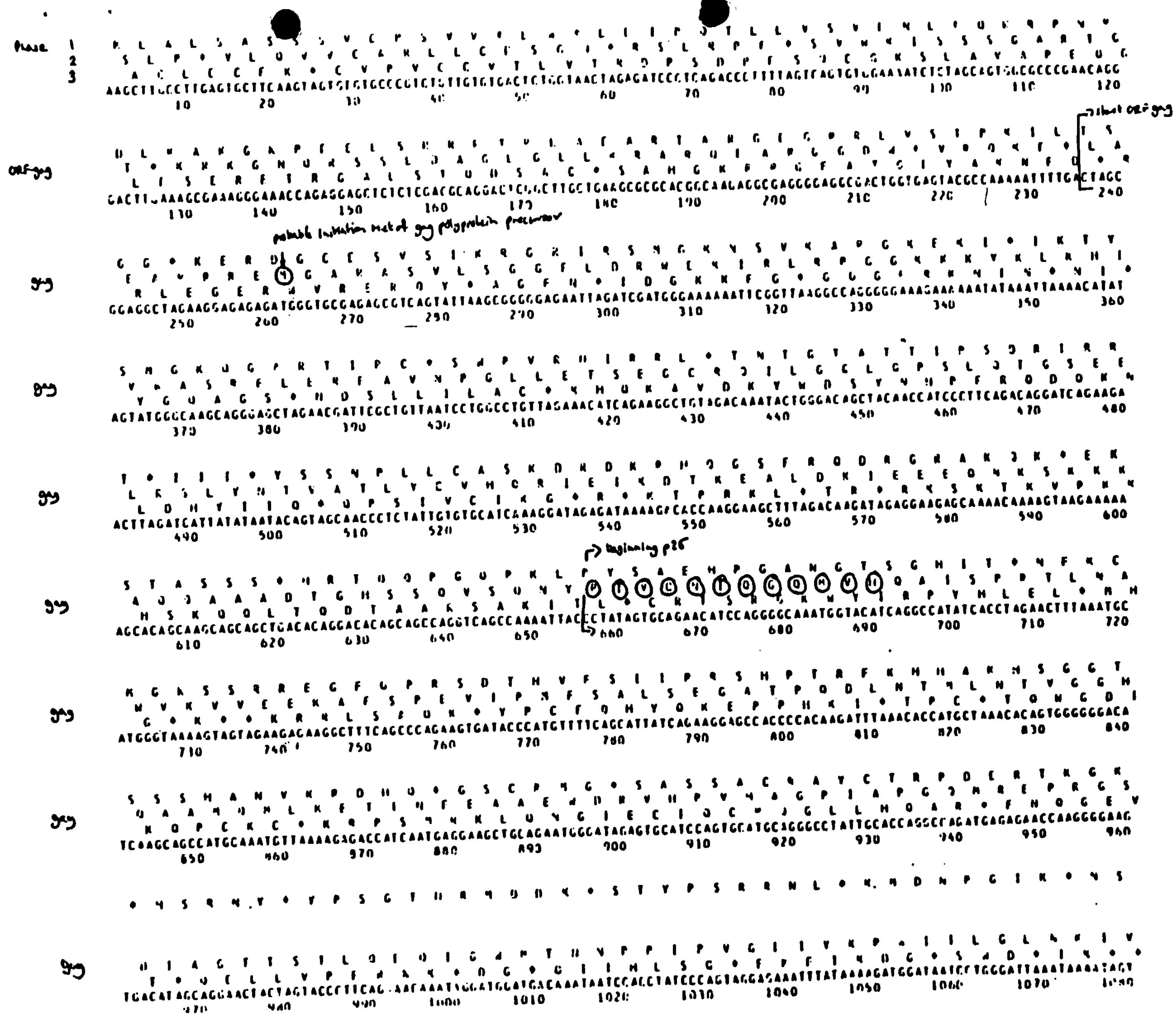


Fig. 3



Phase 1
2
3
ORF-gag
start ORF gag
probable initiation met of gag polyprotein precursor
gag
beginning p25

Fig 4

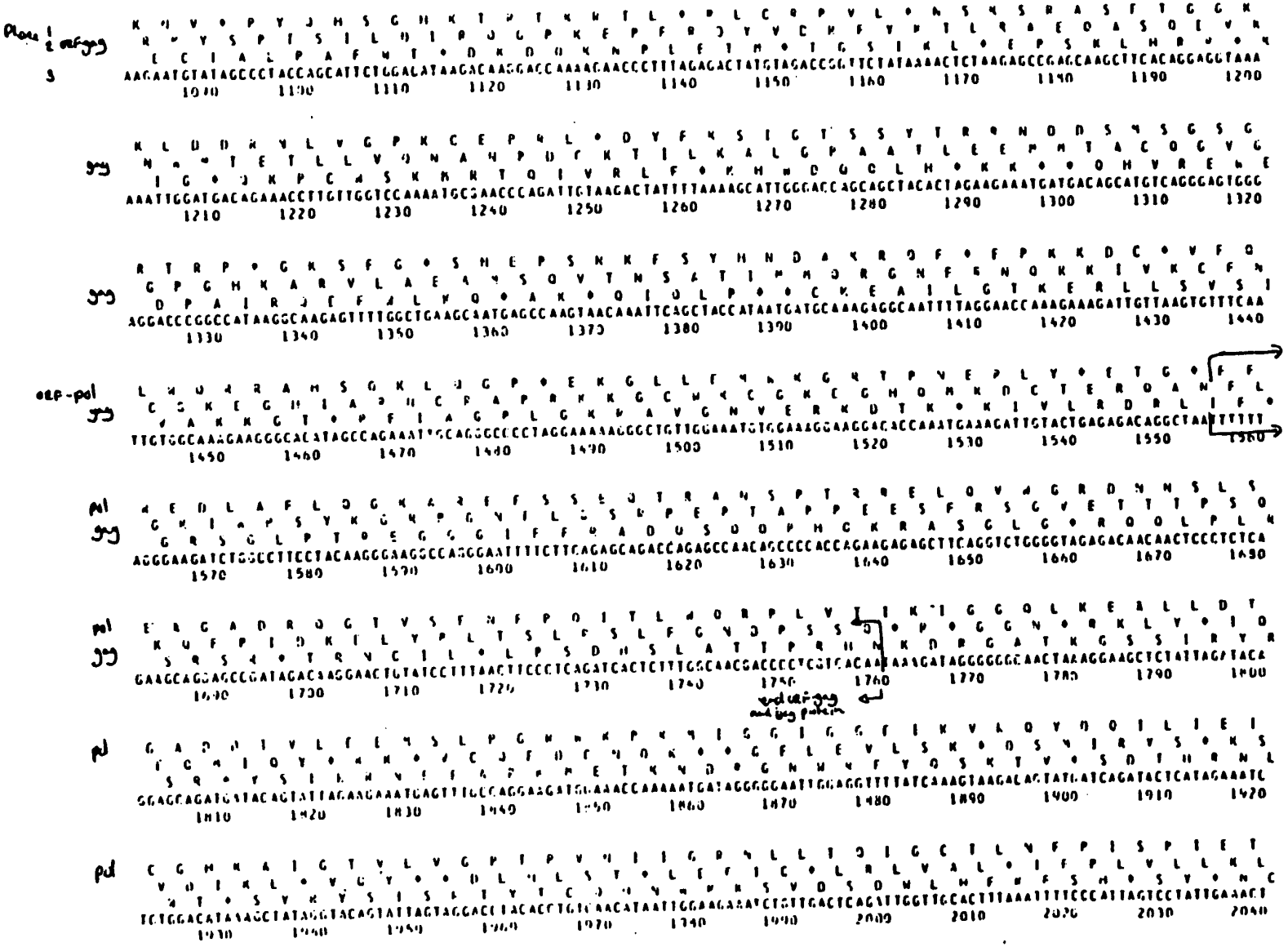


Fig. 5

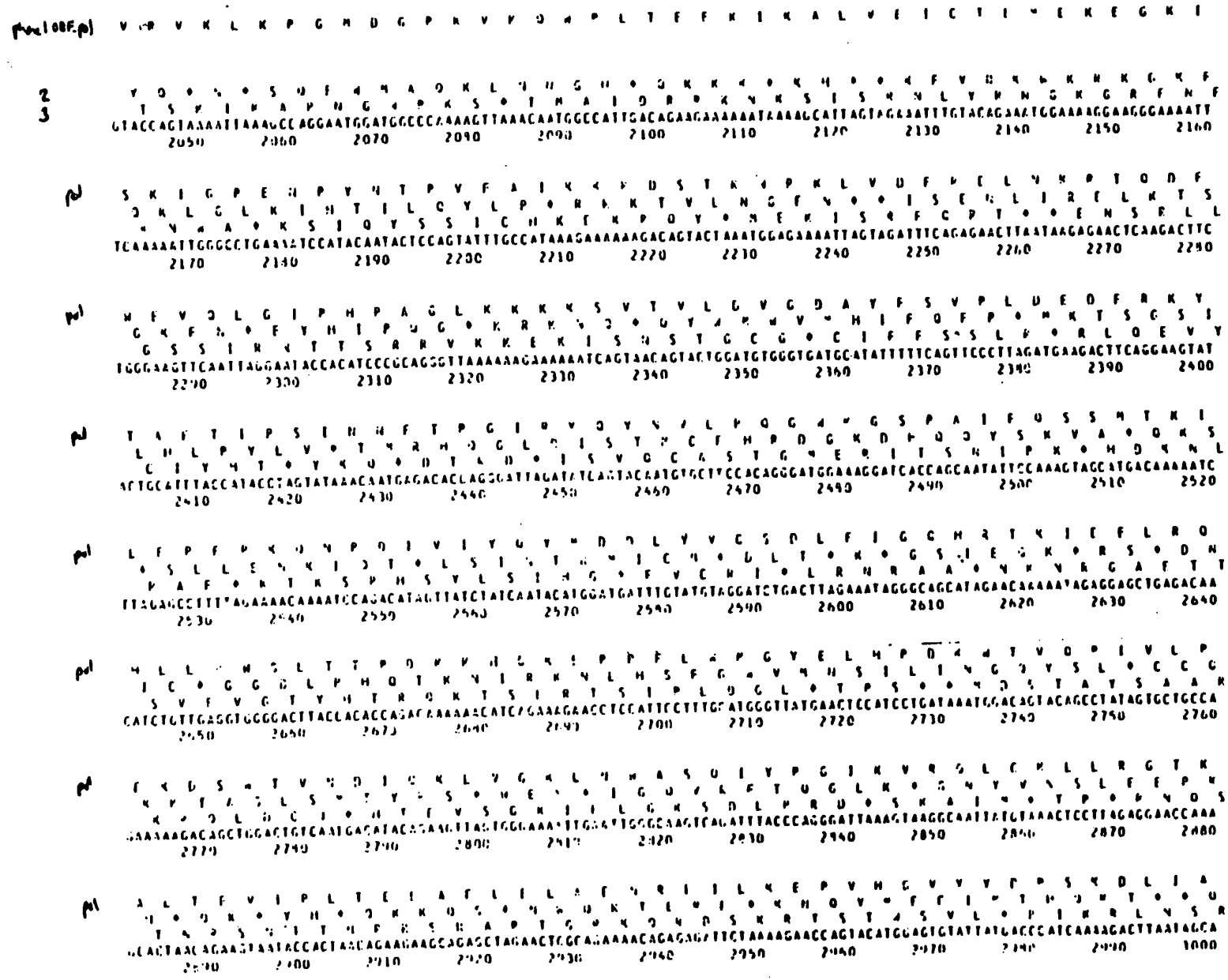


Fig 6

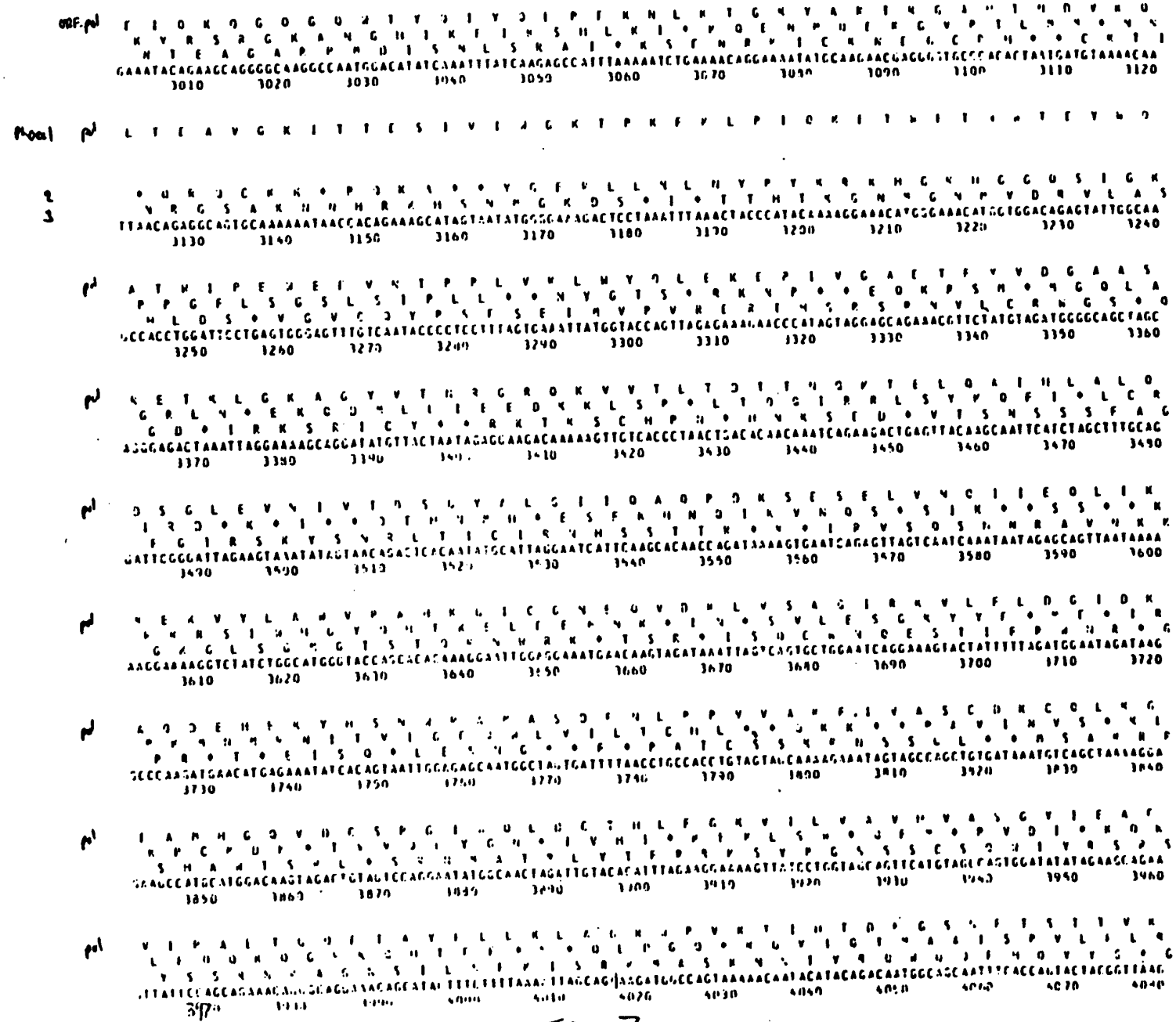


Fig 7

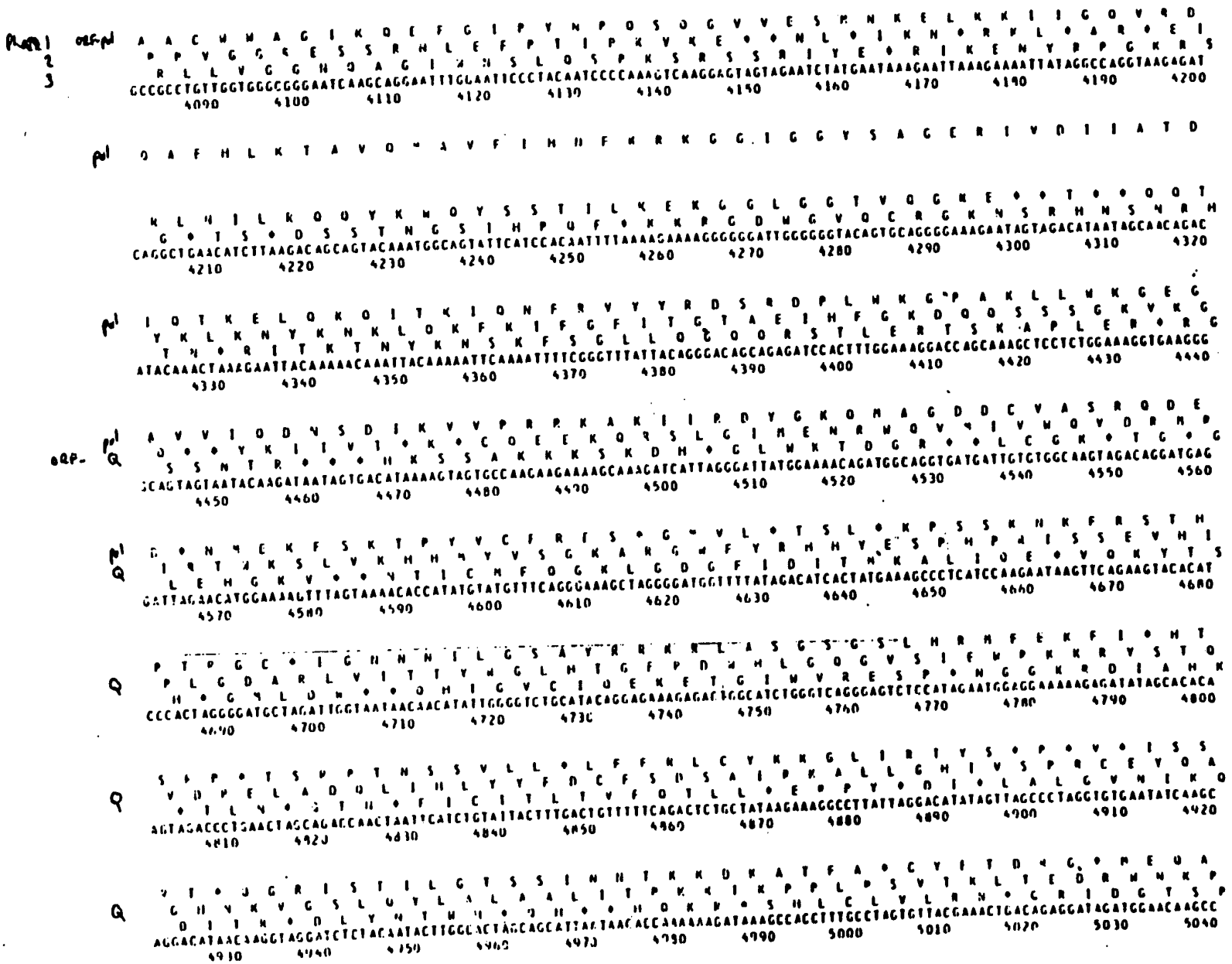


Fig. 8.

end ORF-pol

```
       P E D Q G P Q R E P H N E W I L F L L E E L K N E A V P H F P R I W L H G L G Q
ORF-Q   Q K T K G H R G S H T H V G H • S F • R S L R N A L L D I F L G F G S W A • G H
         R R P R A T E G A T Q • M D T R A F R G A • E • S C • T F S • D L A P W L R A T
       CCAGAAGACCAAGGGCCACAGAGGGAGCCACACAATGAATGGACACTAGAGCTTTTAGAGGAGCTTAAGAATGAAGCTGTTAGACATTTTCCTAGGATTTGGCTCCATGGCTTAGGGCAA
          5050      5060      5070      5080      5090      5100      5110      5120      5130      5140      5150      5160

phase 1  H I Y E T Y G D T W A G V E A I I R I L Q Q L L F I H F R I G C R H S R I G V T
      2   I S M K L M G I L G Q E W K P • • F F C N N C C L S I S E L G V D I A E • A L L
      3    Y L • N L W G Y L G R S G S H N K N S A T T A V Y P F Q N W V S T • Q N R R V S
       CATATCTATGAAACTTATGGGGATACTTGGGCAGGAGTGGAAGCCATAATAAGAATTCTGCAACAACTGCTGTTTATCCATTTCAGAATTGGGTGTCGACATAGCAGAATAGGCGTTACT
          5170      5180      5190      5200      5210      5220      5230      5240      5250      5260      5270      5280

       Q Q R R A R N G A S P S • T R A L E A S F K S A • N C L Y H L L L • K V L L S L

       N R G E Q E W E P V D P R L E P W K H P G S Q P K T A C T T C Y C K K C C F H C
        T E E S K K M S Q • I L D • S P G S I Q E V S L K L L V P L A I V K S V A F I A
       CAACAGAGGAGAGCAAGAAATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAACTGCTTGTACCACTTGCTATTGTAAAAAGTGTTGCTTTCATTG
          5290      5300      5310      5320      5330      5340      5350      5360      5370      5380      5390      5400

       P S L F H N K S L R H L L W Q E E A E T A T K T S S R Q S D S S S F S I K A V S
        Q V C F T T K A L G I S Y G R K K R R Q R R R P P Q G S Q T H C V S L S K Q • V
         K F V S Q Q K P • A S P M A G R S G D S D E D L L K A V R L I K F L Y Q S S K •
       CCAAGTTTGTTTCACAACAAAAGCCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGTAAGT
          5410      5420      5430      5440      5450      5460      5470      5480      5490      5500      5510      5520

       S T C N A T Y T N S N S S I S S S N N N S N S C V V H S N H R I • E N I K T K K
        V H V M Q P I Q I A I A A L V V A I I I A I V V W S I V I I E Y R K I L R Q R K
         Y M • C N L Y K • Q • Q H • • Q • • • Q • L C G P • • S • N I G K Y • D K E K
       AGTACATGTAATGCAACCTATACAAATAGCAATAGCAGCATTAGTAGTAGCAATAATAATAGCAATAGTTGTGTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAAAGAAA
          5530      5540      5550      5560      5570      5580      5590      5600      5610      5620      5630      5640

       M R Q V N • • T N R K S R R Q W Q • F • R R N I S T C G D G G G N G A P C S L G
        I D R L I D R L I E R A E D S G N E S E G E I S A L V E M G V E M G H H A P W D
ORF-env  • T G • L I D • • K E Q K T V A M R V K E K Y Q H L W R W G W K W G T M L L G I
       AATAGACAGGTTAATTGATAGACTAATAGAAAGAGCAGAAGACAGTGGCAATGAGAGTGAAGGAGAAATATCAGCACTTGTGGAGATGGGGGTGGAAATGGGGCACCATGCTCCTTGGGA
          5650      5660      5670      5680      5690      5700      5710      5720      5730      5740      5750      5760
                               └> start ORF-env   possible start of env transcription

       Y • • S V V L D K N C G S Q S I M G Y L C G F K Q P P L Y F V H Q W L K H H I Q
        I D D L • C Y R K I V G H S L L W G T C V E G S N H H S I L C I Q C • S I • V P
env      L M I C S A T E K L W V T V Y Y G V P V W K E A T T T L F C A S D A K A Y D T E
       TATTGATGATCTGTAGTGCTACAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAGGAAGCAACCACCACTCTATTTTGTGCATCAGATGCTAAAGCATATGATACAG
          5770      5780      5790      5800      5810      5820      5830      5840      5850      5860      5870      5880

       R Y I M F G P H M P V Y P Q T P T H K K • Y W • M • Q K I L T C G A P T W • N R
        G T • C L G H T C L C T H R P Q P T • S S I G K C D R K F • H V E K • M G R T D
env      V H N V W A T H A C V P T D P N P Q E V V L V N V T E N F N M W K N D M V E Q M
       AGGTACATAATGTTTGGGCCACACATGCCTGTGTACCCACAGACCCCAACCCACAAGAAGTAGTATTGGTAAATGTGACAGAAAATTTTAACATGTGGAAAAATGACATGGTAGAACAGA
          5890      5900      5910      5920      5930      5940      5950      5960      5970      5980      5990      6000

       C M R I • S V Y G I W A • S H V • N • P H S V L V • S A L I W G W L L I P I V V
        A • G Y N Q I W G S K P K P C C K I N P T L L C V S L K C T D L G N A T N T N S S N
env      H E D I I S L W D Q S L K P C V K L T P L C V T L K C T D L G N A T N T N S S N
       TGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAAGCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAAGTGCACTGATTTGGGGAATGCTACTAATACCAATAGTAGTA
          6010      6020      6030      6040      6050      6060      6070      6080      6090      6100      6110      6120
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Fig 9

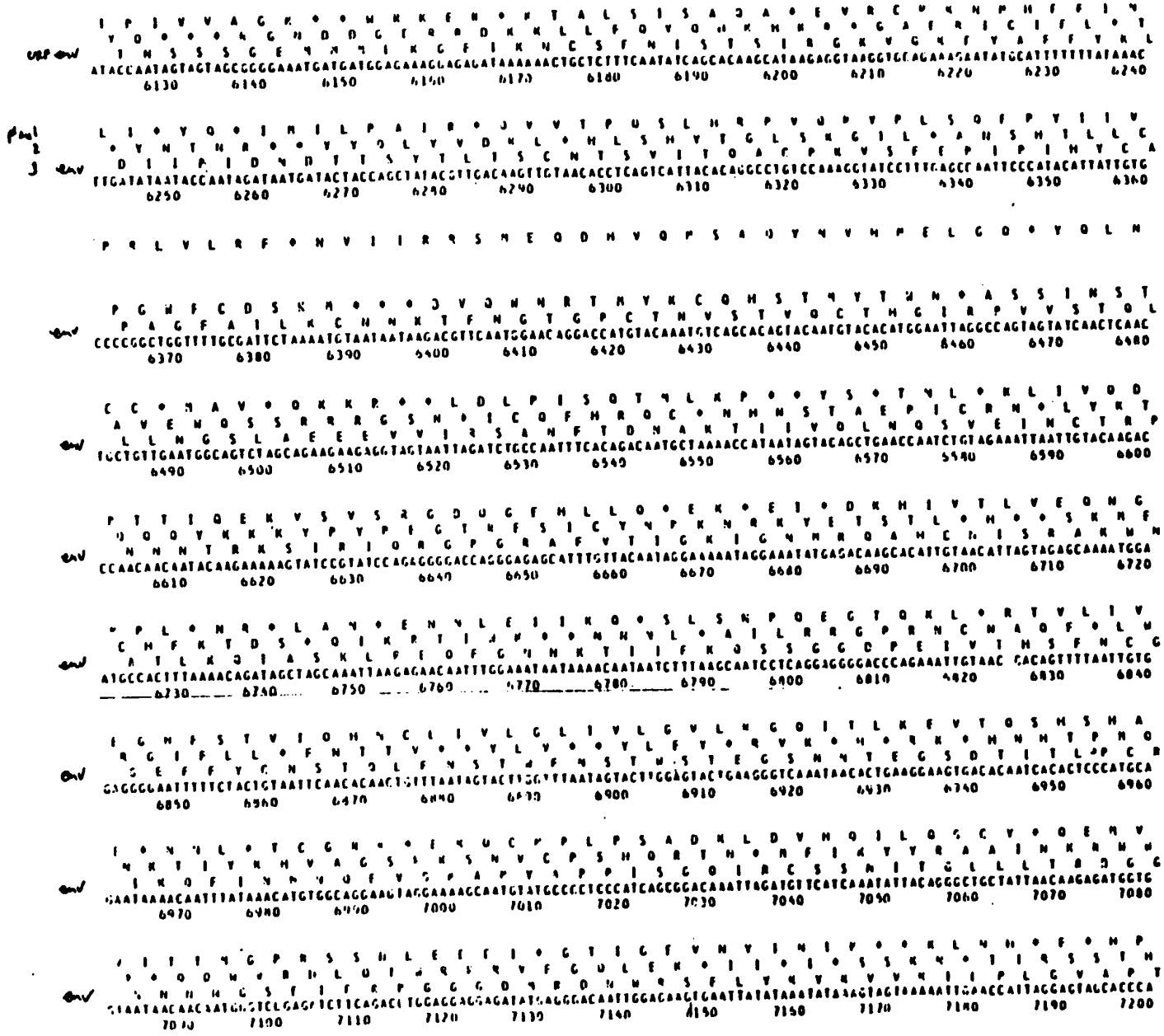


Fig. 10

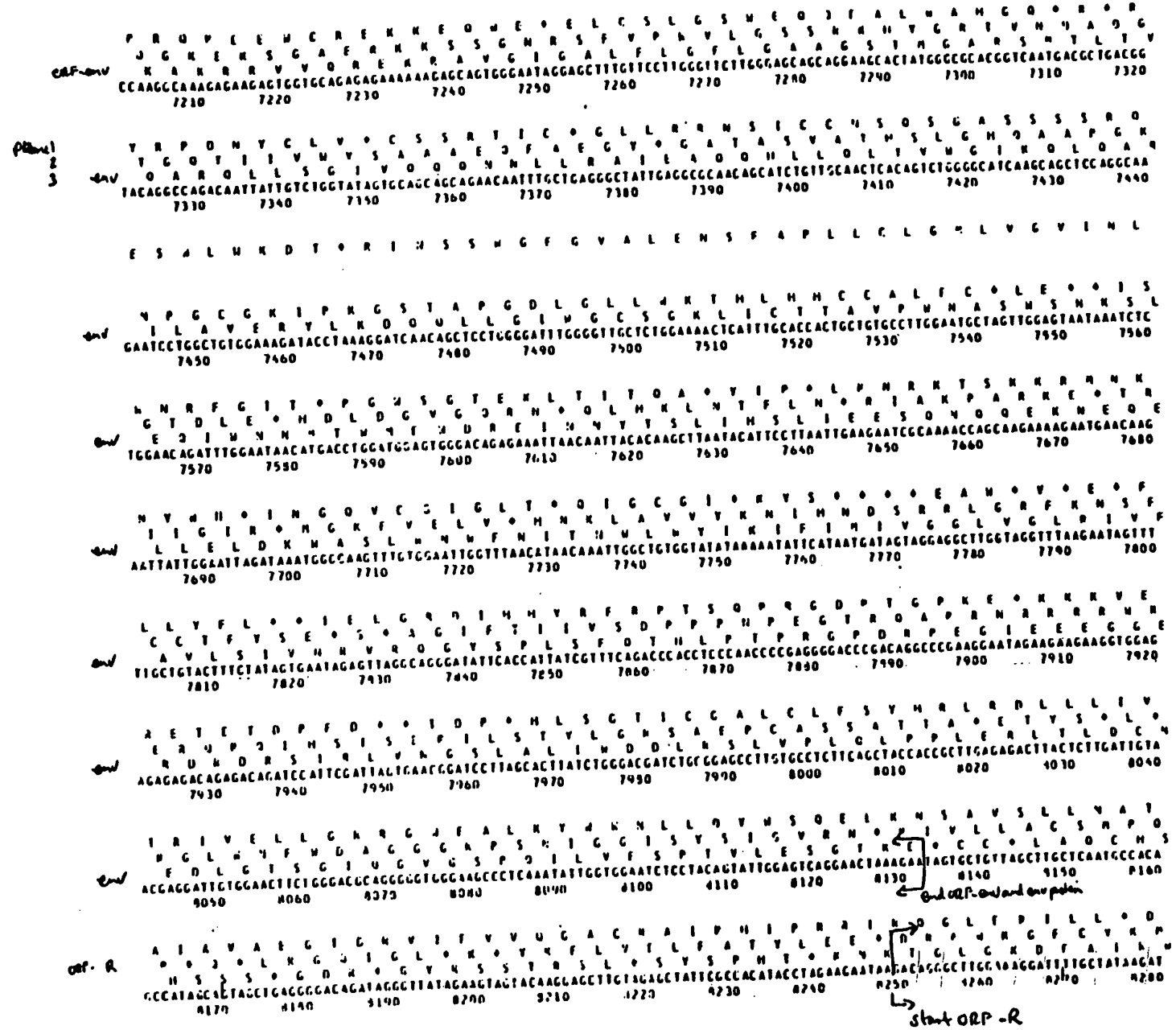
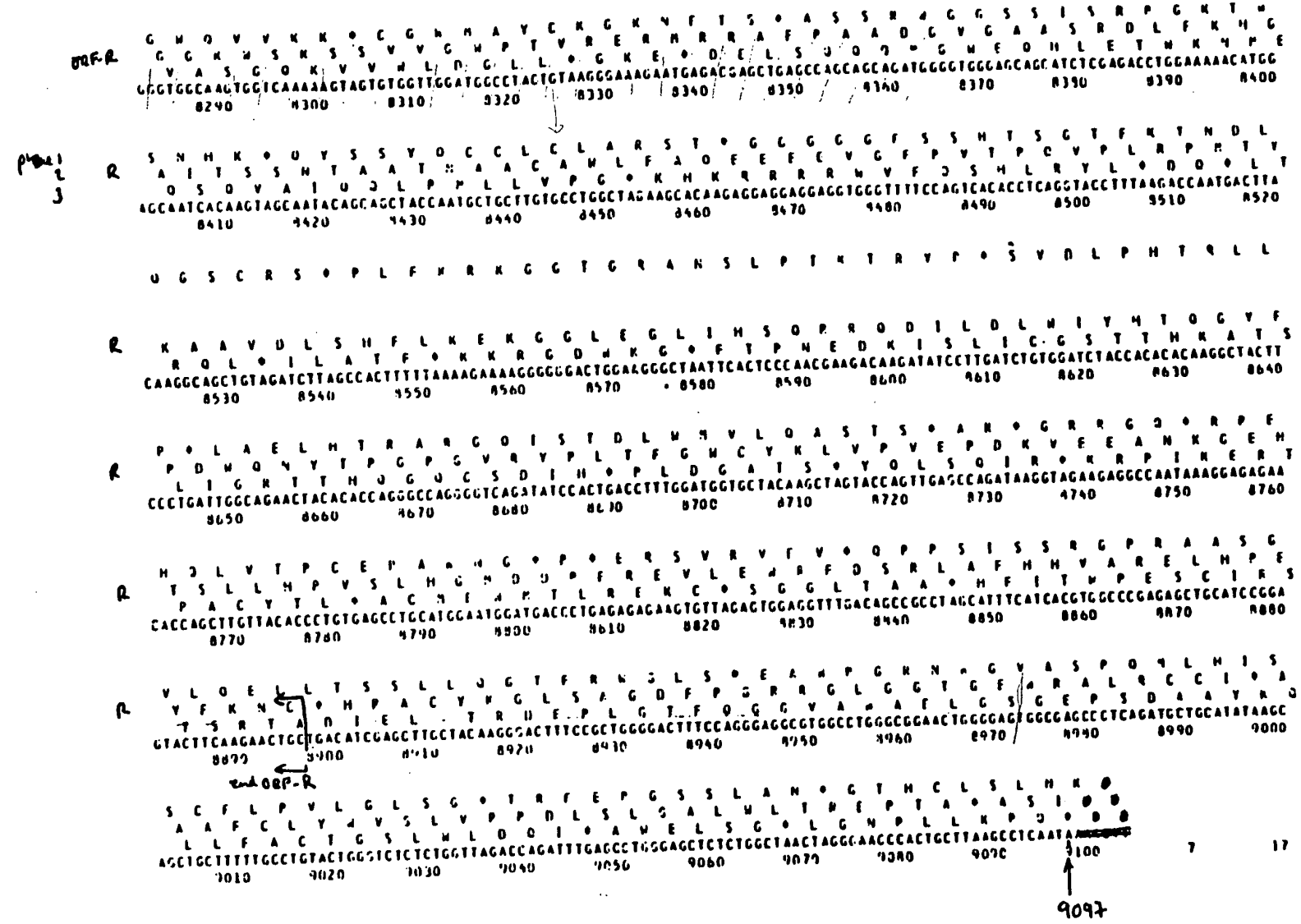


Fig 11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8870
8880
8890
8900
8910
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8930
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8950
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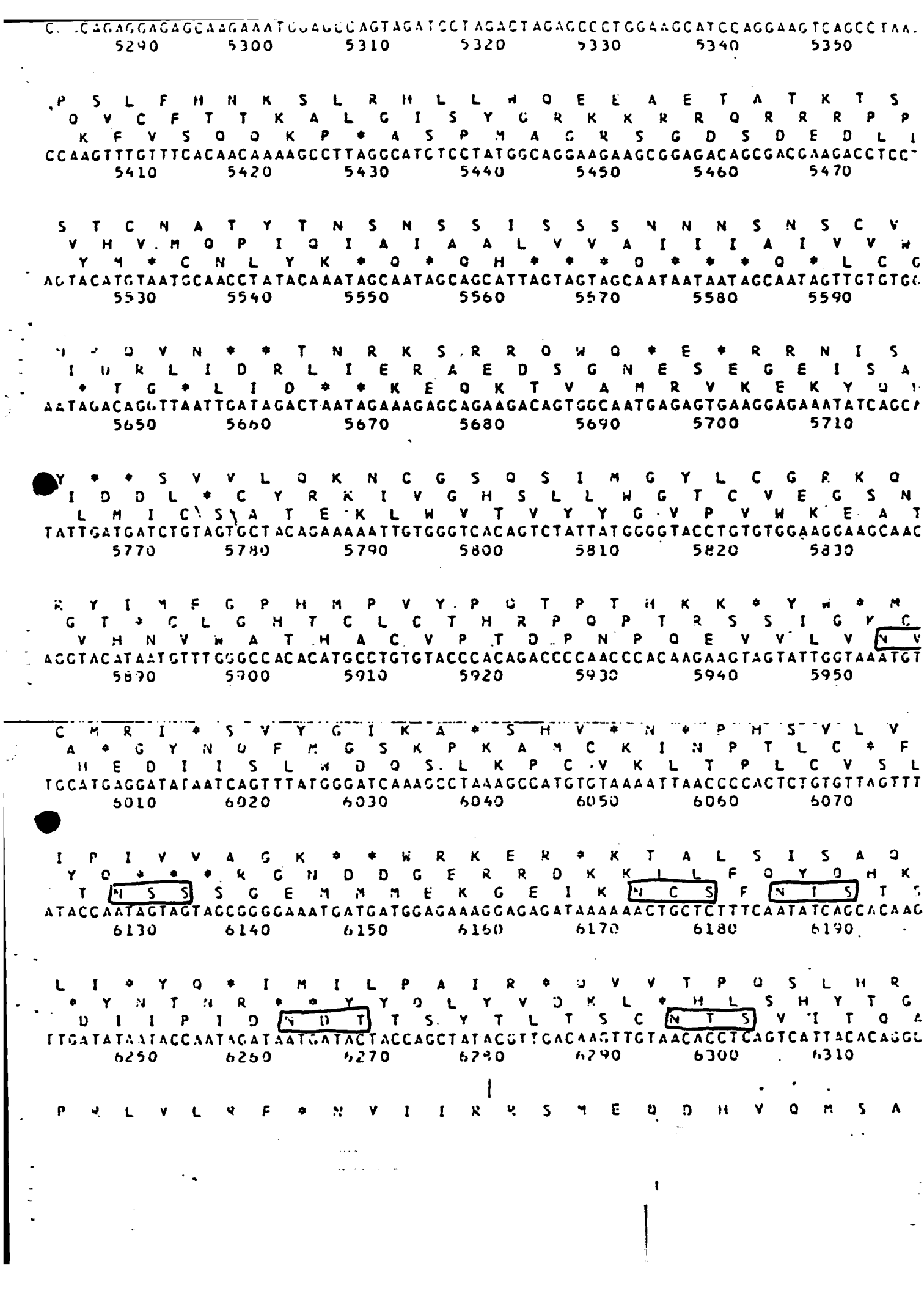
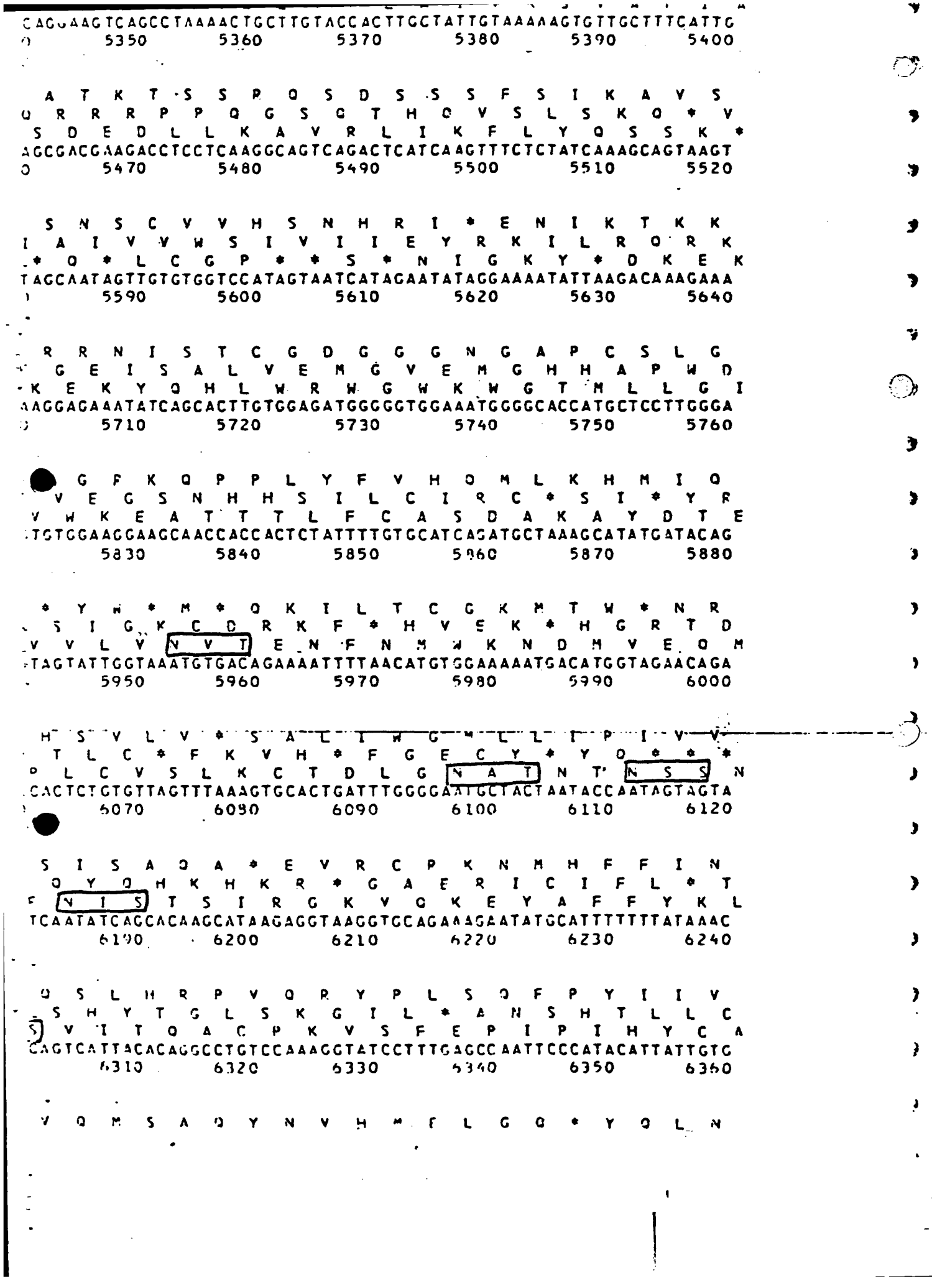
8970
8980
8990
9000
end ORF-R
9097
7
17

fig 12

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Q R R R P P Q G S G T H C V S L S K Q * V
S D E D L L K A V R L I K F L Y Q S S K *
AGCGACGAAGACCTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAGTAAGT
5470 5480 5490 5500 5510 5520
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I A I V V W S I V I I E Y R K I L R Q R K
* Q * L C G P * * S * N I G K Y * D K E K
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R R N I S T C G D G G G N G A P C S L G
G E I S A L V E M G V E M G H H A P W D
K E K Y Q H L W R W G W K W G T M L L G I
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V E G S N H H S I L C I R C * S I * Y R
V W K E A T T T L F C A S D A K A Y D T E
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5830 5840 5850 5960 5870 5880
* Y W * M * Q K I L T C G K M T W * N R
S I G K C D R K F * H V E K * H G R T D
V V L V N V T E N F N M W K N D M V E Q M
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T L C * F K V H * F G E C Y * Y Q * * *
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Q Y Q H K H K R * G A E R I C I F L * T
F N I S T S I R G K V Q K E Y A F F Y K L
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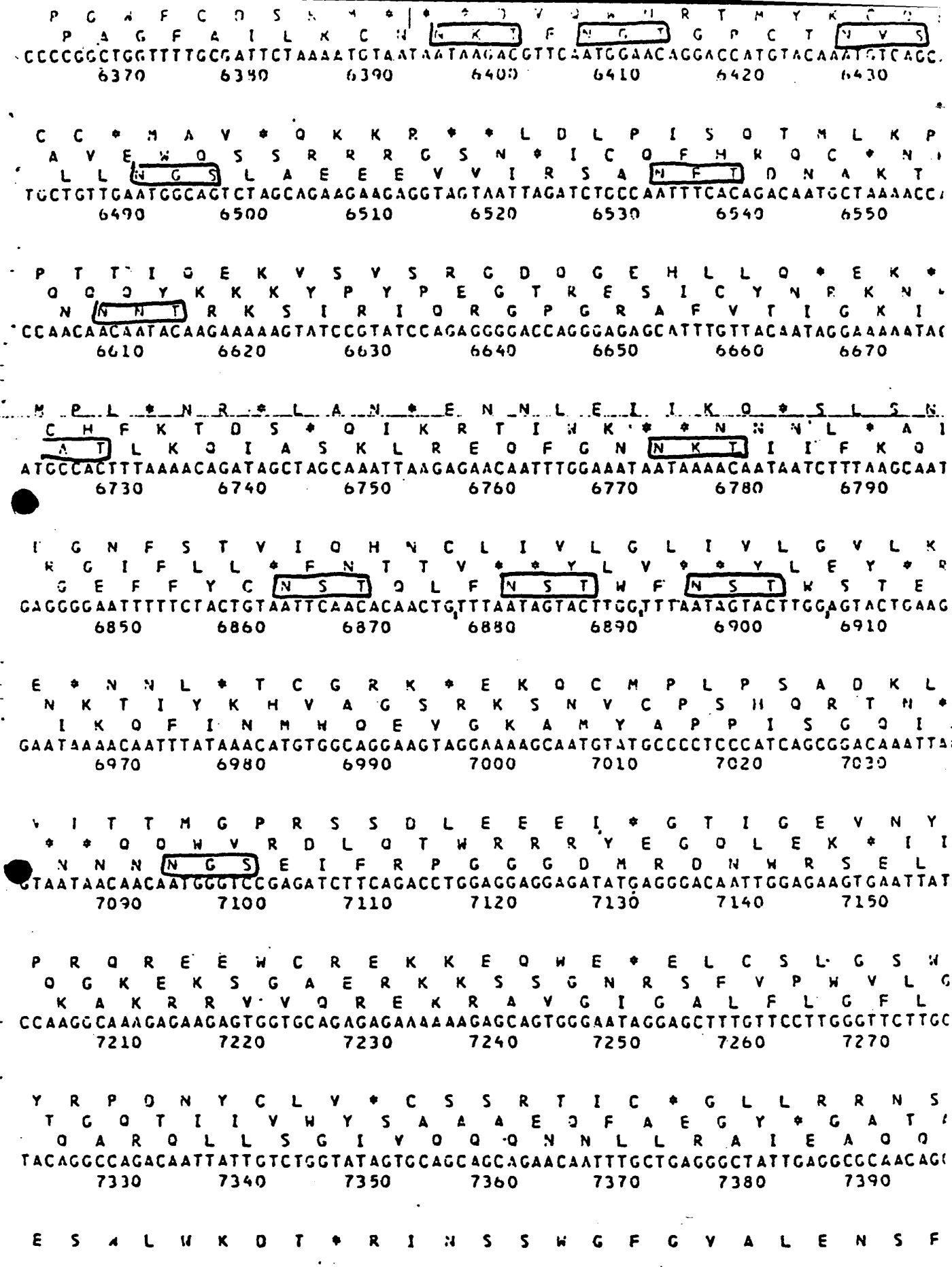

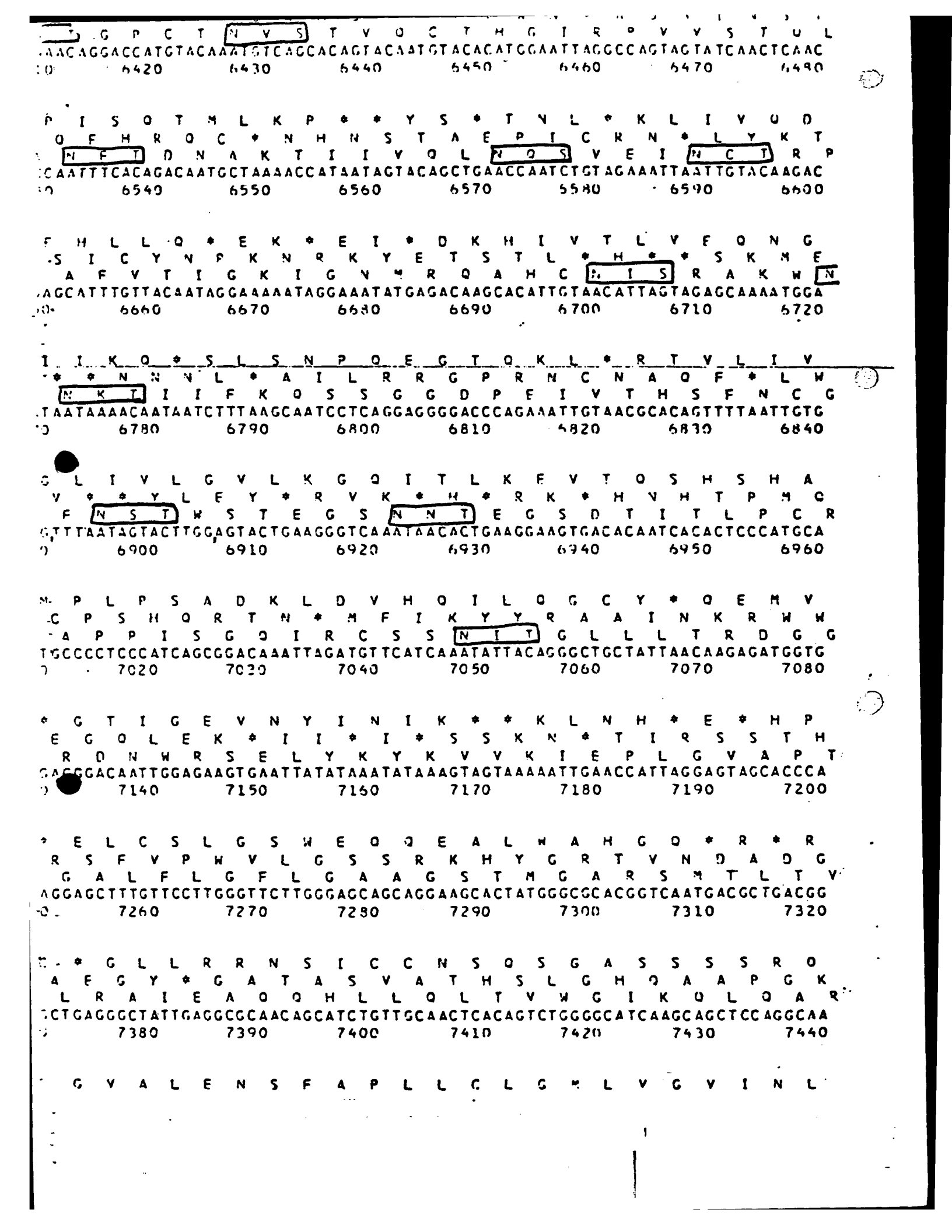
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P I S Q T M L K P * * Y S * T N L * K L I V Q D
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N F T D N A K T I I V Q L N Q S V E I N C T R P
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R D N W R S E L Y K Y K V V K I E P L G V A P T
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10 7140 7150 7160 7170 7180 7190 7200
* E L C S L G S W E Q Q E A L W A H G Q * R * R
R S F V P W V L G S S R K H Y G R T V N D A D G
G A L F L G F L G A A G S T M G A R S M T L T V
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* G L L R R N S I C C N S Q S G A S S S S R Q
A F G Y * G A T A S V A T H S L G H Q A A P G K
L R A I E A Q Q H L L Q L T V W G I K Q L Q A R
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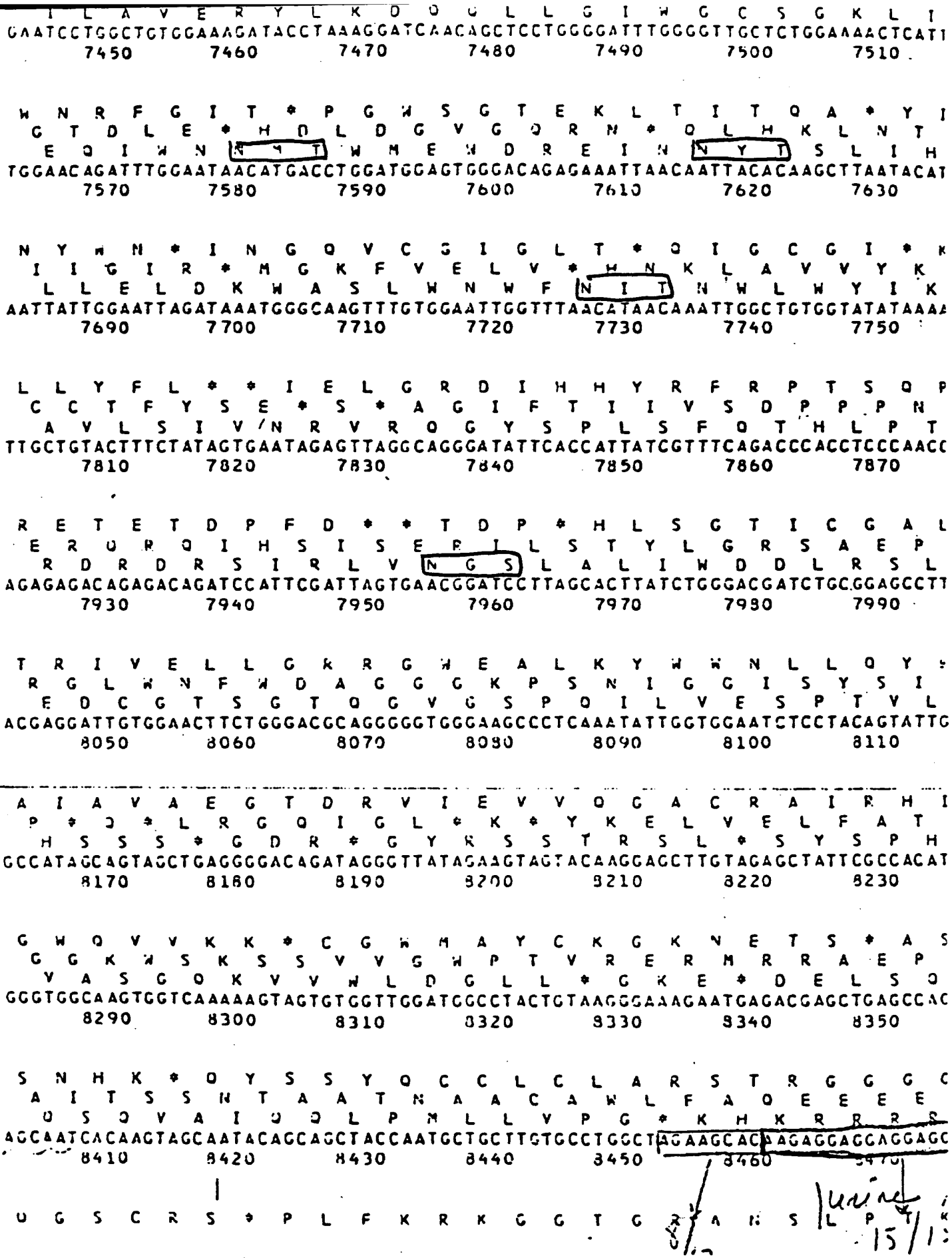
I L A V E R Y L K D Q G L L G I W G C S G K L I
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7450 7460 7470 7480 7490 7500 7510
W N R F G I T * P G W S G T E K L T I T Q A * Y I
G T D L E * H D L D G V G Q R N * Q L H K L N T
E Q I W N N Y T W M E W D R E I N N Y T S L I H
TGGAACAGATTTGGAATAACATGACCTGGATGGAGTGGGACAGAGAAATTAACAATTACACAAGCTTAATACAT
7570 7580 7590 7600 7610 7620 7630
N Y W N * I N G Q V C G I G L T * Q I G C G I * K
I I G I R * M G K F V E L V * H N K L A V V Y K
L L E L D K W A S L W N W F N I T N W L W Y I K
AATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACATAACAAATTGGCTGTGGTATATAAAA
7690 7700 7710 7720 7730 7740 7750
L L Y F L * * I E L G R D I H H Y R F R P T S Q P
C C T F Y S E * S * A G I F T I I V S D P P P N
A V L S I V N R V R Q G Y S P L S F Q T H L P T
TTGCTGTACTTTCTATAGTGAATAGAGTTAGGCAGGGATATTCACCATTATCGTTTCAGACCCACCTCCCAACC
7810 7820 7830 7840 7850 7860 7870
R E T E T D P F D * * T D P * H L S G T I C G A L
E R D R Q I H S I S E P I L S T Y L G R S A E P
R D R D R S I R L V N G S L A L I W D D L R S L
AGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTT
7930 7940 7950 7960 7970 7980 7990
T R I V E L L G K R G W E A L K Y W W N L L Q Y *
R G L W N F W D A G G G K P S N I G G I S Y S I
E D C G T S G T Q G V G S P Q I L V E S P T V L
ACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTACAGTATTG
8050 8060 8070 8080 8090 8100 8110
A I A V A E G T D R V I E V V Q G A C R A I R H I
P * D * L R G Q I G L * K * Y K E L V E L F A T
H S S S * G D R * G Y R S S T R S L * S Y S P H
GCCATAGCAGTAGCTGAGGGGACAGATAGGGTTATAGAAGTAGTACAAGGAGCTTGTAGAGCTATTCGCCACAT
8170 8180 8190 8200 8210 8220 8230
G W Q V V K K * C G W M A Y C K G K N E T S * A S
G G K W S K S S V V G W P T V R E R M R R A E P
V A S G Q K V V W L D G L L * G K E * D E L S Q
GGGTGGCAAGTGGTCAAAAAGTAGTGTGGTTGGATGGCCTACTGTAAGGGAAAGAATGAGACGAGCTGAGCCAC
8290 8300 8310 8320 8330 8340 8350
S N H K * Q Y S S Y Q C C L C L A R S T R G G G C
A I T S S N T A A T N A A C A W L F A Q E E E E
Q S Q V A I Q D L P M L L V P G * K H K R R R R
AGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGCTTGTGCCTGGCTAGAAGCACAAGAGGAGGAGGAGC
8410 8420 8430 8440 8450 8460
U G S C R S * P L F K R K G G T G R A N S L P T

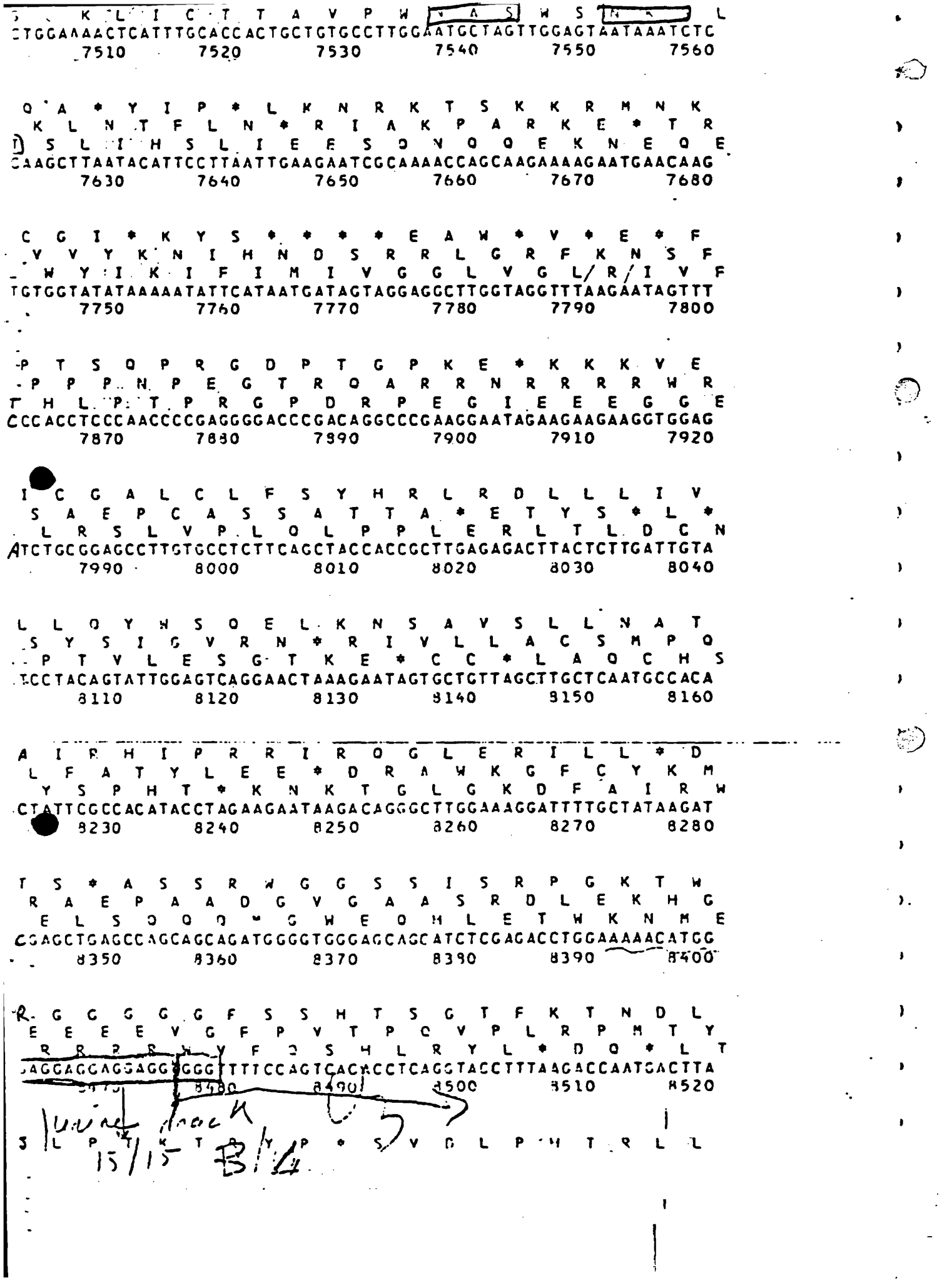
7510 7520 7530 7540 7550 7560
7630 7640 7650 7660 7670 7680
7750 7760 7770 7780 7790 7800
7870 7880 7890 7900 7910 7920
7990 8000 8010 8020 8030 8040
8110 8120 8130 8140 8150 8160
8230 8240 8250 8260 8270 8280
8350 8360 8370 8390 8390 8400
8500 8510 8520

Fig 19

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          10          20          30          40          50          60
AAGCTTGCCT TGAGTGCTTC AAGTAGTGTG TGCCCGTCTG TTGTGTGACT CTGGTAACTA

          70          80          90         100         110         120
GAGATCCCTC AGACCCTTTT AGTCAGTGTG GAAAATCTCT AGCAGTGGCG CCCGAACAGG

         130         140         150         160         170         180
GACTTGAAAG CGAAAGGGAA ACCAGAGGAG CTCTCTCGAC GCAGGACTCG GCTTGCTGAA

         190         200         210         220         230         240
GCGCGCACGG CAAGAGGCGA GGGGAGGCGA CTGGTGAGTA CGCCAAAAAT TTTGACTAGC

         250         260         270         280         290         300
GGAGGCTAGA AGGAGAGAGA TGGGTGCGAG AGCGTCAGTA TTAAGCGGGG GAGAATTAGA

         310         320         330         340         350         360
TCGATGGGAA AAAATTCGGT TAAGGCCAGG GGGAAAGAAA AAATATAAAT TAAAACATAT

         370         380         390         400         410         420
AGTATGGGCA AGCAGGGAGC TAGAACGATT CGCTGTTAAT CCTGGCCTGT TAGAAACATC

         430         440         450         460         470         480
AGAAGGCTGT AGACAAATAC TGGGACAGCT ACAACCATCC CTTCAGACAG GATCAGAAGA

         490         500         510         520         530         540
ACTTAGATCA TTATATAATA CAGTAGCAAC CCTCTATTGT GTGCATCAAA GGATAGAGAT

         550         560         570         580         590         600
AAAAGACACC AAGGAAGCTT TAGACAAGAT AGAGGAAGAG CAAAACAAAA GTAAGAAAAA

         610         620         630         640         650         660
AGCACAGCAA GCAGCAGCTG ACACAGGACA CAGCAGCCAG GTCAGCCAAA ATTACCCTAT

         670         680         690         700         710         720
AGTGCAGAAC ATCCAGGGGC AAATGGTACA TCAGGCCATA TCACCTAGAA CTTTAAATGC

         730         740         750         760         770         780
ATGGGTAAAA GTAGTACAAG AGAAGGCTTT CAGCCCAGAA GTGATACCCA TGTTTTCAGC

         790         800         810         820         830         840
ATTATCAGAA GGAGCCACCC CACAAGATTT AAACACCATG CTAAACACAG TGGGGGGACA

         850         860         870         880         890         900
TCAAGCAGCC ATGCAAATGT TAAAAGAGAC CATCAATGAG GAAGCTGCAG AATGGGATAG

         910         920         930         940         950         960
AGTGCATCCA GTGCATGCAG GGCCTATTGC ACCAGGCCAG ATGAGAGAAC CAAGGGGAAG

         970         980         990        1000        1010        1020
TGACATAGCA GGAACTACTA GTACCCTTCA GGAACAAATA GGATGGATGA CAAATAATCC

        1030        1040        1050        1060        1070        1080
ACCTATCCCA GTAGGAGAAA TTTATAAAAG ATGGATAATC CTGGGATTAA ATAAAATAGT

        1090        1100        1110        1120        1130        1140
```

Fig 9D

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AAGAATGTAT AGCCCTACCA GCATTCTGGA CATAAGACAA GGACCAAAAG AACCCTTTAG

      1150        1160        1170        1180        1190        1200
AGACTATGTA GACCGGTTCT ATAAAACTCT AAGAGCCGAG CAAGCTTCAC AGGAGGTAAA

      1210        1220        1230        1240        1250        1260
AAATTGGATG ACAGAAACCT TGTTGGTCCA AAATGCGAAC CCAGATTGTA AGACTATTTT

      1270        1280        1290        1300        1310        1320
AAAAGCATTG GGACCAGCAG CTACACTAGA AGAAATCATG ACAGCATGTC AGGGAGTGGG

      1330        1340        1350        1360        1370        1380
AGGACCCGGC CATAAGGCAA GAGTTTTGGC TGAAGCAATG AGCCAAGTAA CAAATTCAGC

      1390        1400        1410        1420        1430        1440
TACCATAATG ATGCAAAGAG GCAATTTTAG GAACCAAAGA AAGATTGTTA AGTGTTTCAA

      1450        1460        1470        1480        1490        1500
TTGTGGCAAA GAAGGGCACA TAGCCAGAAA TTGCAGGGCC CCTAGGAAAA AGGGCTGTTG

      1510        1520        1530        1540        1550        1560
GAAATGTGGA AAGGAAGGAC ACCAAATGAA AGATTGTACT GAGAGACAGG CTAATTTTTT

      1570        1580        1590        1600        1610        1620
AGGGAAGATC TGGCCTTCCT ACAAGGGAAG GCCAGGGAAT TTTCTTCAGA GCAGACCAGA

      1630        1640        1650        1660        1670        1680
GCCAACAGCC CCACCAGAAG AGAGCTTCAG GTCTGGGGTA GAGACAACAA CTCCCTCTCA

      1690        1700        1710        1720        1730        1740
GAAGCAGGAG CCGATAGACA AGGAACTGTA TCCTTTAACT TCCCTCAGAT CACTCTTTGG

      1750        1760        1770        1780        1790        1800
CAACGACCCC TCGTCACAAT AAAGATAGGG GGGCAACTAA AGGAAGCTCT ATTAGATACA

      1810        1820        1830        1840        1850        1860
GGAGCAGATG ATACAGTATT AGAAGAAATG AGTTTGCCAG GAAGATGGAA ACCAAAAATG

      1870        1880        1890        1900        1910        1920
ATAGGGGGAA TTGGAGGTTT TATCAAAGTA AGACAGTATG ATCAGATACT CATAGAAATC

      1930        1940        1950        1960        1970        1980
TGTGGACATA AAGCTATAGG TACAGTATTA GTAGGACCTA CACCTGTCAA CATAATTGGA

      1990        2000        2010        2020        2030        2040
AGAAATCTGT TGACTCAGAT TGGTTGCACT TTAAATTTTC CCATTAGTCC TATTGAAACT

      2050        2060        2070        2080        2090        2100
GTACCAGTAA AATTAAAGCC AGGAATGGAT GGCCCAAAAG TTAAACAATG GCCATTGACA

      2110        2120        2130        2140        2150        2160
GAAGAAAAAA TAAAAGCATT AGTAGAAATT TGTACAGAAA TGGAAAAGGA AGGGAAAATT

      2170        2180        2190        2200        2210        2220
TCAAAAATTG GGCCTGAAAA TCCATACAAT ACTCCAGTAT TTGCCATAAA GAAAAAAGAC

      2230        2240        2250        2260        2270        2280
AGTACTAAAT GGAGAAAATT AGTAGATTTC AGAGAACTTA ATAAGAGAAC TCAAGACTTC

      2290        2300        2310        2320        2330        2340
TGGGAAGTTC AATTAGGAAT ACCACATCCC GCAGGGTTAA AAAAGAAAAA ATCAGTAACA
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GTACTGAATG TGGGTGATGC ATATTTTTCA GTTCCCTTAG ATGAAGACTT CAGGAACTAT

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      2410        2420        2430        2440        2450        2460
ACTGCATTTA CCATACCTAG TATAAACAAT GAGACACCAG GGATTAGATA TCAGTACAAT

      2470        2480        2490        2500        2510        2520
GTGCTTCCAC AGGGATGGAA AGGATCACCA GCAATATTCC AAAGTAGCAT GACAAAAATC

      2530        2540        2550        2560        2570        2580
TTAGAGCCTT TTAGAAAACA AAATCCAGAC ATAGTTATCT ATCAATACAT GGATGATTTG

      2590        2600        2610        2620        2630        2640
TATGTAGGAT CTGACTTAGA AATAGGGCAG CATAGAACAA AAATAGAGGA GCTGAGACAA

      2650        2660        2670        2680        2690        2700
CATCTGTTGA GGTGGGGACT TACCACACCA GACAAAAAAC ATCAGAAAGA ACCTCCATTC

      2710        2720        2730        2740        2750        2760
CTTTGGATGG GTTATGAACT CCATCCTGAT AAATGGACAG TACAGCCTAT AGTGCTGCCA

      2770        2780        2790        2800        2810        2820
GAAAAAGACA GCTGGACTGT CAATGACATA CAGAAGTTAG TGGGAAAATT GAATTGGGCA

      2830        2840        2850        2860        2870        2880
AGTCAGATTT ACCCAGGGAT TAAAGTAAGG CAATTATGTA AACTCCTTAG AGGAACCAAA

      2890        2900        2910        2920        2930        2940
GCACTAACAG AAGTAATACC ACTAACAGAA GAAGCAGAGC TAGAACTGGC AGAAAACAGA

      2950        2960        2970        2980        2990        3000
GAGATTCTAA AAGAACCAGT ACATGGAGTG TATTATGACC CATCAAAAGA CTTAATAGCA

      3010        3020        3030        3040        3050        3060
GAAATACAGA AGCAGGGGCA AGGCCAATGG ACATATCAAA TTTATCAAGA GCCATTTAAA

      3070        3080        3090        3100        3110        3120
AATCTGAAAA CAGGAAAATA TGCAAGAACG AGGGGTGCCC ACACTAATGA TGTAAAACAA

      3130        3140        3150        3160        3170        3180
TTAACAGAGG CAGTGCAAAA AATAACCACA GAAAGCATAG TAATATGGGG AAAGACTCCT

      3190        3200        3210        3220        3230        3240
AAATTTAAAC TACCCATACA AAAGGAAACA TGGGAAACAT GGTGGACAGA GTATTGGCAA

      3250        3260        3270        3280        3290        3300
GCCACCTGGA TTCCTGAGTG GGAGTTTGTC AATACCCCTC CTTTAGTGAA ATTATGGTAC

      3310        3320        3330        3340        3350        3360
CAGTTAGAGA AAGAACCCAT AGTAGGAGCA GAAACGTTCT ATGTAGATGG GGCAGCTAGC

      3370        3380        3390        3400        3410        3420
AGGGAGACTA AATTAGGAAA AGCAGGATAT GTTACTAATA GAGGAAGACA AAAAGTTGTC

      3430        3440        3450        3460        3470        3480
ACCCTAACTG ACACAACAAA TCAGAAGACT GAGTTACAAG CAATTCATCT AGCTTTGCAG

      3490        3500        3510        3520        3530        3540
GATTCGGGAT TAGAAGTAAA TATAGTAACA GACTCACAAT ATGCATTAGG AATCATTCAA

      3550        3560        3570        3580        3590        3600
GCACAACCAG ATAAAAGTGA ATCAGAGTTA GTCAATCAAA TAATAGAGCA GTTAATAAAA

      3610        3620        3630        3640        3650        3660
```

Fig 22

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      3730        3740        3750        3760        3770        3780
GCCCAAGATG AACATGAGAA ATATCACAGT AATTGGAGAG CAATGGCTAG TGATTTTAAC

      3790        3800        3810        3820        3830        3840
CTGCCACCTG TAGTAGCAAA AGAAATAGTA GCCAGCTGTG ATAAATGTCA GCTAAAAGGA

      3850        3860        3870        3880        3890        3900
GAAGCCATGC ATGGACAAGT AGACTGTAGT CCAGGAATAT GGCAACTAGA TTGTACACAT

      3910        3920        3930        3940        3950        3960
TTAGAAGGAA AAGTTATCCT GGTAGCAGTT CATGTAGCCA GTGGATATAT AGAAGCAGAA

      3970        3980        3990        4000        4010        4020
GTTATTCCAG CAGAAACAGG GCAGGAAACA GCATACTTTC TTTTAAAATT AGCAGGAAGA

      4030        4040        4050        4060        4070        4080
TGGCCAGTAA AAACAATACA TACAGACAAT GGCAGCAATT TCACCAGTAC TACGGTTAAG

      4090        4100        4110        4120        4130        4140
GCCGCCTGTT GGTGGGCGGG AATCAAGCAG GAATTTGGAA TTCCCTACAA TCCCCAAAGT

      4150        4160        4170        4180        4190        4200
CAAGGAGTAG TAGAATCTAT GAATAAAGAA TTAAAGAAAA TTATAGGCCA GGTAAGAGAT

      4210        4220        4230        4240        4250        4260
CAGGCTGAAC ATCTTAAGAC AGCAGTACAA ATGGCAGTAT TCATCCACAA TTTTAAAAGA

      4270        4280        4290        4300        4310        4320
AAAGGGGGGA TTGGGGGGTA CAGTGCAGGG GAAAGAATAG TAGACATAAT AGCAACAGAC

      4330        4340        4350        4360        4370        4380
ATACAAACTA AAGAATTACA AAAACAAATT ACAAAAATTC AAAATTTTCG GGTTTATTAC

      4390        4400        4410        4420        4430        4440
AGGGACAGCA GAGATCCACT TTGGAAAGGA CCAGCAAAGC TCCTCTGGAA AGGTGAAGGG

      4450        4460        4470        4480        4490        4500
GCAGTAGTAA TACAAGATAA TAGTGACATA AAAGTAGTGC CAAGAAGAAA AGCAAAGATC

      4510        4520        4530        4540        4550        4560
ATTAGGGATT ATGGAAAACA GATGGCAGGT GATGATTGTG TGGCAAGTAG ACAGGATGAG

      4570        4580        4590        4600        4610        4620
GATTAGAACA TGGAAAAGTT TAGTAAAACA CCATATGTAT GTTTCAGGGA AAGCTAGGGG

      4630        4640        4650        4660        4670        4680
ATGGTTTTAT AGACATCACT ATGAAAGCCC TCATCCAAGA ATAAGTTCAG AAGTACACAT

      4690        4700        4710        4720        4730        4740
CCCACTAGGG GATGCTAGAT TGGTAATAAC AACATATTGG GGTCTGCATA CAGGAGAAAG

      4750        4760        4770        4780        4790        4800
AGACTGGCAT CTGGGTCAGG GAGTCTCCAT AGAATGGAGG AAAAAGAGAT ATAGCACACA

      4810        4820        4830        4840        4850        4860
AGTAGACCCT GAACTAGCAG ACCAACTAAT TCATCTGTAT TACTTTGACT GTTTTTCAGA

      4870        4880        4890        4900        4910        4920
```

Fig. 23

2510

AGGACATAAC AAGGTAGGAT CTCTACAATA CTTGGCACTA GCAGCATTAA TAACACCAAA

4990 5000 5010 5020 5030 5040
AAAGATAAAG CCACCTTTGC CTAGTGTTAC GAAACTGACA GAGGATAGAT GGAACAAGCC

5050 5060 5070 5080 5090 5100
CCAGAAGACC AAGGGCCACA GAGGGAGCCA CACAATGAAT GGACACTAGA GCTTTTAGAG

5110 5120 5130 5140 5150 5160
GAGCTTAAGA ATGAAGCTGT TAGACATTTT CCTAGGATTT GGCTCCATGG CTTAGGGCAA

5170 5180 5190 5200 5210 5220
CATATCTATG AAACTTATGG GGATACTTGG GCAGGAGTGG AAGCCATAAT AAGAATTCTG

5230 5240 5250 5260 5270 5280
CAACAACTGC TGTTTATCCA TTTCAGAATT GGGTGTCGAC ATAGCAGAAT AGGCGTTACT

5290 5300 5310 5320 5330 5340
CAACAGAGGA GAGCAAGAAA TGGAGCCAGT AGATCCTAGA CTAGAGCCCT GGAAGCATCC

5350 5360 5370 5380 5390 5400
AGGAAGTCAG CCTAAAACTG CTTGTACCAC TTGCTATTGT AAAAAGTGTT GCTTTCATTG

5410 5420 5430 5440 5450 5460
CCAAGTTTGT TTCACAACAA AAGCCTTAGG CATCTCCTAT GGCAGGAAGA AGCGGAGACA

5470 5480 5490 5500 5510 5520
GCGACGAAGA CCTCCTCAAG GCAGTCAGAC TCATCAAGTT TCTCTATCAA AGCAGTAAGT

5530 5540 5550 5560 5570 5580
AGTACATGTA ATGCAACCTA TACAAATAGC AATAGCAGCA TTAGTAGTAG CAATAATAAT

5590 5600 5610 5620 5630 5640
AGCAATAGTT GTGTGGTCCA TAGTAATCAT AGAATATAGG AAAATATTAA GACAAAGAAA

5650 5660 5670 5680 5690 5700
AATAGACAGG TTAATTGATA GACTAATAGA AAGAGCAGAA GACAGTGGCA ATGAGAGTGA

5710 5720 5730 5740 5750 5760
AGGAGAAATA TCAGCACTTG TGGAGATGGG GGTGGAAATG GGGCACCATG CTCCTTGGGA

5770 5780 5790 5800 5810 5820
TATTGATGAT CTGTAGTGCT ACAGAAAAAT TGTGGGTCAC AGTCTATTAT GGGGTACCTG

5830 5840 5850 5860 5870 5880
TGTGGAAGGA AGCAACCACC ACTCTATTTT GTGCATCAGA TGCTAAAGCA TATGATACAG

5890 5900 5910 5920 5930 5940
AGGTACATAA TGTTTGGGCC ACACATGCCT GTGTACCCAC AGACCCCAAC CCACAAGAAG

5950 5960 5970 5980 5990 6000
TAGTATTGGT AAATGTGACA GAAAATTTTA ACATGTGGAA AAATGACATG GTAGAACAGA

6010 6020 6030 6040 6050 6060
TGCATGAGGA TATAATCAGT TTATGGGATC AAAGCCTAAA GCCATGTGTA AAATTAACCC

6070 6080 6090 6100 6110 6120
CACTCTGTGT TAGTTTAAAG TGCACTGATT TGGGGAATGC TACTAATACC AATAGTAGTA

6130 6140 6150 6160 6170 6180

TCAATATCAG CACAAGCATA AGAGGTAAGG TGCAGAAAGA ATATGCATTT TTTTATAAAC

6250 6260 6270 6280 6290 6300
TTGATATAAT ACCAATAGAT AATGATACTA CCAGCTATAC GTTGACAAGT TGTAACACCT

6310 6320 6330 6340 6350 6360
CAGTCATTAC ACAGGCCTGT CCAAAGGTAT CCTTTGAGCC AATTCCCATA CATTATTGTG

6370 6380 6390 6400 6410 6420
CCCCGGCTGG TTTTGCGATT CTAAAATGTA ATAATAAGAC GTTCAATGGA ACAGGACCAT

6430 6440 6450 6460 6470 6480
GTACAAATGT CAGCACAGTA CAATGTACAC ATGGAATTAG GCCAGTAGTA TCAACTCAAC

6490 6500 6510 6520 6530 6540
TGCTGTTGAA TGGCAGTCTA GCAGAAGAAG AGGTAGTAAT TAGATCTGCC AATTTCACAG

6550 6560 6570 6580 6590 6600
ACAATGCTAA AACCATAATA GTACAGCTGA ACCAATCTGT AGAAATTAAT TGTACAAGAC

6610 6620 6630 6640 6650 6660
CCAACAACAA TACAAGAAAA AGTATCCGTA TCCAGAGGGG ACCAGGGAGA GCATTTGTTA

6670 6680 6690 6700 6710 6720
CAATAGGAAA AATAGGAAAT ATGAGACAAG CACATTGTAA CATTAGTAGA GCAAAATGGA

6730 6740 6750 6760 6770 6780
ATGCCACTTT AAAACAGATA GCTAGCAAAT TAAGAGAACA ATTTGGAAAT AATAAAACAA

6790 6800 6810 6820 6830 6840
TAATCTTTAA GCAATCCTCA GGAGGGGACC CAGAAATTGT AACGCACAGT TTTAATTGTG

6850 6860 6870 6880 6890 6900
GAGGGGAATT TTTCTACTGT AATTCAACAC AACTGTTTAA TAGTACTTGG TTTAATAGTA

6910 6920 6930 6940 6950 6960
CTTGGAGTAC TGAAGGGTCA AATAACACTG AAGGAAGTGA CACAATCACA CTCCCATGCA

6970 6980 6990 7000 7010 7020
GAATAAAACA ATTTATAAAC ATGTGGCAGG AAGTAGGAAA AGCAATGTAT GCCCCTCCCA

7030 7040 7050 7060 7070 7080
TCAGCGGACA AATTAGATGT TCATCAAATA TTACAGGGCT GCTATTAACA AGAGATGGTG

7090 7100 7110 7120 7130 7140
GTAATAACAA CAATGGGTCC GAGATCTTCA GACCTGGAGG AGGAGATATC AGGGACAATT

7150 7160 7170 7180 7190 7200
GGAGAAGTGA ATTATATAAA TATAAAGTAG TAAAAATTGA ACCATTAGGA GTAGCACCCA

7210 7220 7230 7240 7250 7260
CCAAGGCAAA GAGAAGAGTG GTGCAGAGAG AAAAAAGAGC AGTGGGAATA GGAGCTTTGT

7270 7280 7290 7300 7310 7320
TCCTTGGGTT CTTGGGAGCA GCAGGAAGCA CTATGGGCGC ACGGTCAATG ACGCTGACGG

7330 7340 7350 7360 7370 7380
TACAGGCCAG ACAATTATTG TCTGGTATAG TGCAGCAGCA GAACAATTTG CTGAGGGCTA

7390 7400 7410 7420 7430 7440

GAATCCTGGC TGTGGAAAGA TACCTAAAGG ATCAACAGCT CCTGGGGATT TGGGGTTGCT

7510 7520 7530 7540 7550 7560
CTGGAAAACT CATTTGCACC ACTGCTGTGC CTTGGAATGC TAGTTGGAGT AATAAATCTC

7570 7580 7590 7600 7610 7620
TGGAACAGAT TTGGAATAAC ATGACCTGGA TGGAGTGGGA CAGAGAAATT AACAATTACA

7630 7640 7650 7660 7670 7680
CAAGCTTAAT ACATTCCTTA ATTGAAGAAT CGCAAAACCA GCAAGAAAAG AATGAACAAG

7690 7700 7710 7720 7730 7740
AATTATTGGA ATTAGATAAA TGGGCAAGTT TGTGGAATTG GTTTAACATA ACAAATTGGC

7750 7760 7770 7780 7790 7800
TGTGGTATAT AAAAATATTC ATAATGATAG TAGGAGGCTT GGTAGGTTTA AGAATAGTTT

7810 7820 7830 7840 7850 7860
TTGCTGTACT TTCTATAGTG AATAGAGTTA GGCAGGGATA TTCACCATTA TCGTTTCAGA

7870 7880 7890 7900 7910 7920
CCCACCTCCC AACCCCGAGG GGACCCGACA GGCCCGAAGG AATAGAAGAA GAAGGTGGAG

7930 7940 7950 7960 7970 7980
AGAGAGACAG AGACAGATCC ATTCGATTAG TGAACGGATC CTTAGCACTT ATCTGGGACG

7990 8000 8010 8020 8030 8040
ATCTGCGGAG CCTTGTGCCT CTTCAGCTAC CACCGCTTGA GAGACTTACT CTTGATTGTA

8050 8060 8070 8080 8090 8100
ACGAGGATTG TGGAACTTCT GGGACGCAGG GGGTGGGAAG CCCTCAAATA TTGGTGGAAT

8110 8120 8130 8140 8150 8160
CTCCTACAGT ATTGGAGTCA GGAACTAAAG AATAGTGCTG TTAGCTTGCT CAATGCCACA

8170 8180 8190 8200 8210 8220
GCCATAGCAG TAGCTGAGGG GACAGATAGG GTTATAGAAG TAGTACAAGG AGCTTGTAGA

8230 8240 8250 8260 8270 8280
GCTATTCGCC ACATACCTAG AAGAATAAGA CAGGGCTTGG AAAGGATTTT GCTATAAGAT

8290 8300 8310 8320 8330 8340
GGGTGGCAAG TGGTCAAAAA GTAGTGTGGT TGGATGGCCT ACTGTAAGGG AAAGAATGAG

8350 8360 8370 8380 8390 8400
ACGAGCTGAG CCAGCAGCAG ATGGGGTGGG AGCAGCATCT CGAGACCTGG AAAAACATGG

8410 8420 8430 8440 8450 8460
AGCAATCACA AGTAGCAATA CAGCAGCTAC CAATGCTGCT TGTGCCTGGC TAGAAGCACA

8470 8480 8490 8500 8510 8520
AGAGGAGGAG GAGGTGGGTT TTCCAGTCAC ACCTCAGGTA CCTTTAAGAC CAATGACTTA

8530 8540 8550 8560 8570 8580
CAAGGCAGCT GTAGATCTTA GCCACTTTTT AAAAGAAAAG GGGGGACTGG AAGGGCTAAT

8590 8600 8610 8620 8630 8640
TCACTCCCAA CGAAGACAAG ATATCCTTGA TCTGTGGATC TACCACACAC AAGGCTACTT

8650 8660 8670 8680 8690 8700

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      8710        8720        8730        8740        8750        8760
GTGCTACAAG CTAGTACCAG TTGAGCCAGA TAAGGTAGAA GAGGCCAATA AAGGAGAGAA

      8770        8780        8790        8800        8810        8820
CACCAGCTTG TTACACCCTG TGAGCCTGCA TGGAATGGAT GACCCTGAGA GAGAAGTGTT

      8830        8840        8850        8860        8870        8880
AGAGTGGAGG TTTGACAGCC GCCTAGCATT TCATCACGTG GCCCGAGAGC TGCATCCGGA

      8890        8900        8910        8920        8930        8940
GTACTTCAAG AACTGCTGAC ATCGAGCTTG CTACAAGGGA CTTTCCGCTG GGGACTTTCC

      8950        8960        8970        8980        8990        9000
AGGGAGGCGT GGCCTGGGCG GAACTGGGGA GTGGCGAGCC CTCAGATGCT GCATATAAGC

      9010        9020        9030        9040        9050        9060
AGCTGCTTTT TGCCTGTACT GGGTCTCTCT GGTTAGACCA GATTTGAGCC TGGGAGCTCT

      9070        9080        9090        9100           0           0
CTGGCTAACT AGGGAACCCA CTGCTTAAGC CTCAATAAAG CTT
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Fig 26